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THE AXIS OF ROTATION AT THE ANKLE JOINT IN MAN. ITS INFLUENCE UPON THE FORM OF THE TALUS AND THE MOBILITY OF THE FIBULA

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THE FORM OF THE TALUS

The upper articular surface of the talus is customarily described as wedge-shaped, its width diminishing from front to back. Measurements indicate that the majority of tali do, in fact, show some narrowing of this surface, but that the triangular facet at its lateral margin—articulating with the inferior transverse tibio-fibular ligament—often gives a misleading impression as to the true reduction in width (Inkster, 1927). A simple index of the degree of 'wedging' of any particular talus is obtained if this reduction (x) is divided by the length of the trochlear surface (y).

To assess the variations of 'wedging' in different specimens, 164 cartilage-covered tali—nine from fresh amputations, the remainder from dissecting-room subjects—were examined. Twelve proved unsuitable for detailed study, mainly due to osteoarthritic changes which obliterated the contours, and this paper is therefore based upon analysis of 152 specimens. For obvious reasons no dried bones are included in this series.

For convenience, the widths of the trochlear surface of each talus were measured at right angles to the medial edge; the anterior measurement was taken at the level of the front of the lateral edge, and the posterior measurement at the posterior limit of the medial articular surface. To measure the length of the trochlear surface a line was taken, parallel to its medial edge, from the point where the medial side of the triangular facet meets the posterior articular margin. The distance between the anterior and posterior limits of the articular cartilage was measured along this line.

The tali showed great variation in the degree of 'wedging', the fraction $100x/y$ ranging from 0 to 14. An attempt was made to correlate this variation with the degrees of mobility of the corresponding fibulae. The expectation was that a parallel-sided talus would be associated with a relatively fixed fibula, while a highly 'wedged' talus would require a fibula which could rotate about a vertical axis to accommodate the talus in all positions from full dorsiflexion to full plantar-flexion. Although rotation of the fibula can be demonstrated in the living body and in the preserved cadaver it is impossible in the latter to assess the range of movement. The superior tibio-fibular joint was therefore examined in each limb to assess the degree of mobility of the fibula.

THE SUPERIOR TIBIO-FIBULAR JOINT

This joint was found to vary considerably, both in form and inclination to the horizontal. However, in most cases it conformed to one of the following three general types:

Type I. The area of articular surface on the tibia is large. The surface is plane, approximately circular, nearly horizontal and usually underlying a projecting canopy of bone. Forty-one specimens with an area of articular surface greater than 20 sq.mm. were classified as type I. The majority (thirty-two tali) had an inclination to the horizontal of less than 30°.

Type II. The area of articular surface is moderately large. The surface is elliptical in shape with a curved profile fitting into a grooved facet on the head of the fibula. This type is frequently in communication with the knee joint via the synovial bursa deep to the popliteus tendon. Forty specimens were classified as type II.

Type III. The area of articular surface is small. The surface is plane but often irregular, and steeply inclined to the horizontal. Forty-two specimens with an area of articular surface less than 15 sq.mm. were classified as type III. The majority (thirty-six tali) had an inclination of more than 30° to the horizontal.

The remaining twenty-nine joints could not be assigned to any of the above groups. Many were plane joints but intermediate in area; others showed arthritic changes which made reliable measurement impossible.

By analogy with other joints, e.g. the superior radio-humeral articulation, it was considered that type I joints would be associated with freely mobile fibulae capable of undergoing axial rotation, and that type III joints would allow little axial rotation, although gliding movements in a vertical plane could take place.

It was found impracticable to attempt an assessment of the mobility of type II joints from simple measurements of area and angle of inclination. Movements at this type of joint are complex; the upper end of the fibula glides in an antero-lateral direction, its path being determined by the attachment of the interosseous membrane. This movement is associated with axial rotation of the lower end. The criteria applied to joints of types I and III to determine mobility in terms of area and steepness of joint surface are clearly inadequate here, since the movement of the head of the fibula is not one of simple rotation. For this reason, type II joints were excluded from the series when assessment was made of the range of mobility of the fibulae.

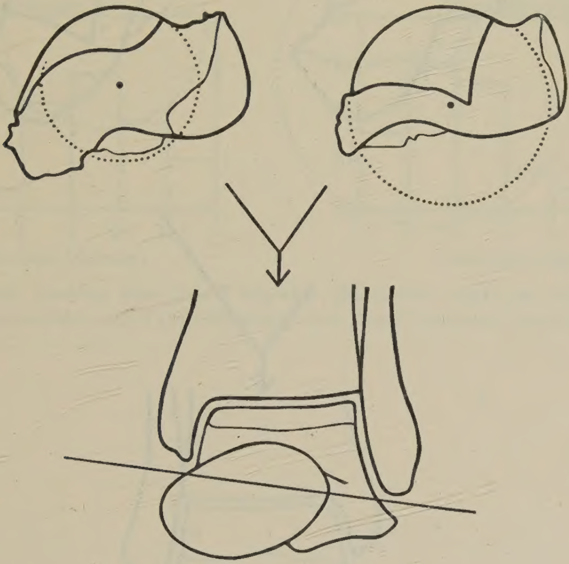
Analysis showed that no correlation existed between the mobility of the fibula and the degree of 'wedging' of the corresponding talus. Contrary to expectation, it was not unusual to find that an extremely 'wedged' talus belonged to a limb where the nature of the superior tibio-fibular joint indicated that the fibula was immobile, while many parallel-sided tali were associated with freely mobile fibulae. It seemed probable, therefore, that the variations in both the mobility of the fibula and the form of the talus might result from differences in the manner of movement of the talus in different individuals. A new study of the talus was accordingly undertaken with regard to the axis about which the bone rotates in dorsiflexion and plantar-flexion.

THE AXIS AT THE ANKLE JOINT

The axis at this joint is usually stated to be horizontal or transverse (Cunningham, 1943). Examination of the lateral and medial profiles of the trochlear surface of the talus indicates that this is rarely the case.

The lateral profile (Pl. 1, fig. 1) is almost always an arc of a true circle, and in all positions of the talus the axis of rotation must pass through the centre of this circle.

The medial profile, on the other hand, is compounded of the arcs of two circles of differing radii (Pl. 1, fig. 2). The axis of rotation is therefore a changing one. The anterior third of the medial profile is part of a circle whose radius is *less* than that of the lateral profile; in dorsiflexion, therefore, when this part of the talus is uppermost in the ankle mortice, in contact with the horizontal lower end of the tibia (Volkov, 1904), the axis of the ankle joint passes through the centre of the large lateral circle and that of the small medial circle and consequently is inclined downwards and laterally ('the dorsiflexion axis') (Text-fig. 1).



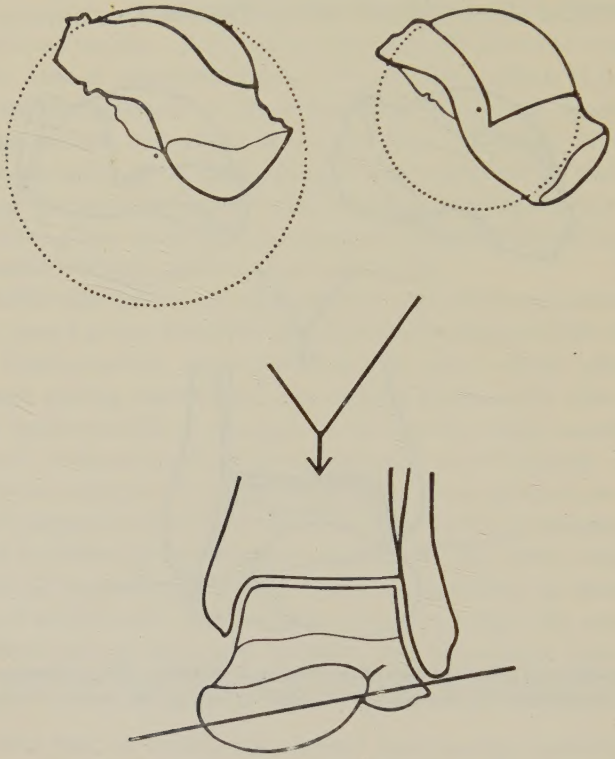
Text-fig. 1. The medial and lateral profiles of the talus in dorsiflexion. The inclination of the dorsiflexion axis (downwards and laterally) is obtained by joining the centres of rotation.

The posterior two-thirds of the medial profile is part of a circle whose radius is *greater* than that of the lateral profile; in plantar-flexion, therefore, the axis of the ankle joint is inclined downwards and medially ('the plantar-flexion axis') (Text-fig. 2).

Thus the axis of the ankle joint is not a fixed horizontal one, but is inclined downwards and laterally during dorsiflexion, downwards and medially during plantar-flexion. The change-over appears to occur within a few degrees of the neutral position of the talus (Pl. 1, fig. 2). The short intermediate curve that is transitional between the anterior and posterior arcs of the medial profile is cycloidal in form.

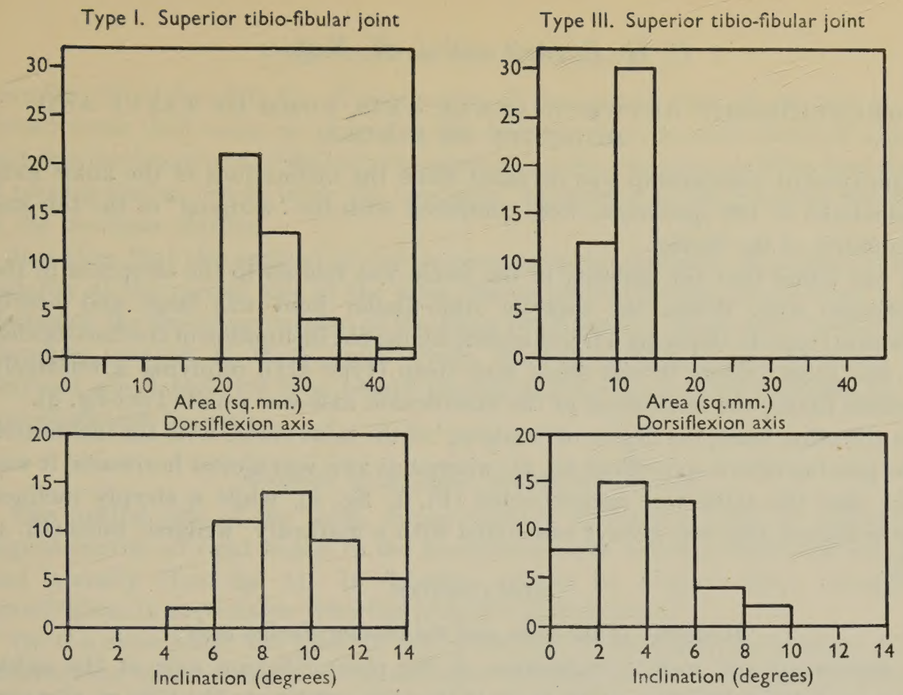
The shape of the talus in respect of the curvatures of the trochlear surface appears to be independent of sex and age. Medial and lateral profiles conforming to the adult pattern have been observed in six foetal limbs, ranging in age from 11 weeks (crown-rump length 50 mm.) to 28 weeks (crown-rump length 200 mm.); four were examined by gross dissection, two by serial sections (Pl. 1, figs. 5, 6).

The inclination of the ankle axis shows wide variations. It is approximately horizontal throughout the dorsiflexion phase in some ankles (5 %) and throughout the plantar-flexion phase in others (12 %). Rarely, the medial and lateral profiles form arcs of identical circles (Pl. 1, figs. 3, 4); the axis then remains horizontal throughout both phases of movement (3 % of tali). In the last instance the upper articular surface of the talus may be said to resemble part of a cylinder, in contrast to the appearance of the more usual type which may be expressed as consisting of two truncated cones lying side by side, apex to base, a concept which agrees in many respects with that described by Goodsir (1869).

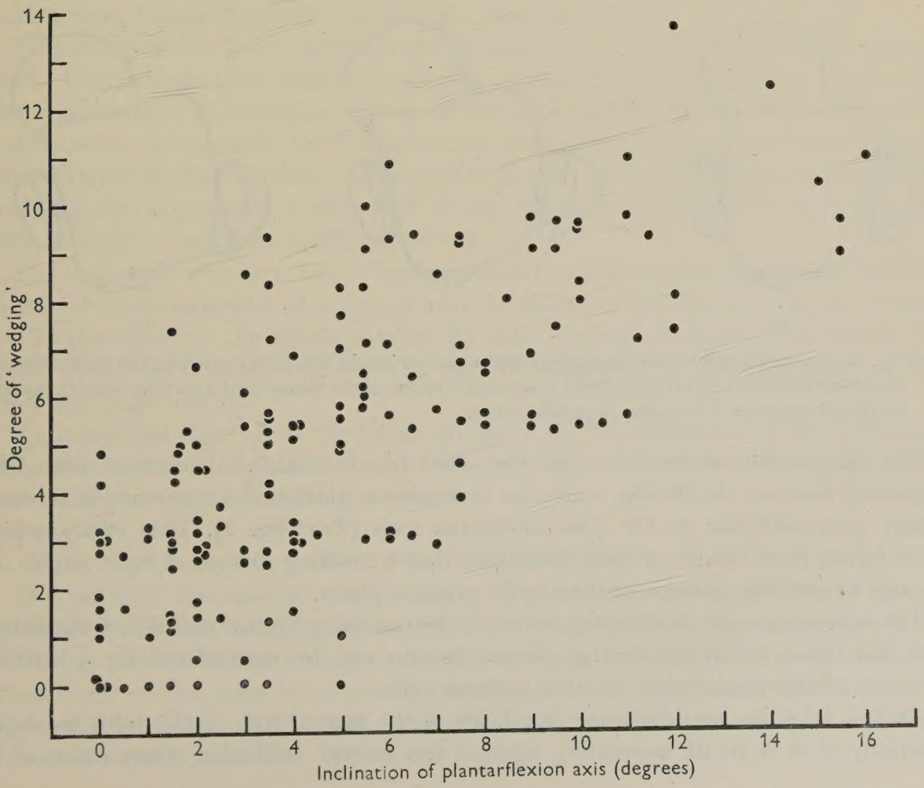


Text-fig. 2. The medial and lateral profiles of the talus in plantar-flexion. The inclination of the plantar-flexion axis (downwards and medially) is obtained by joining the centres of rotation.

Eleven non-European tali were also examined, but these have not been included in the present series as they were not cartilage-covered specimens. The profiles of these bones suggest a typical 'changing' axis, as described above; in the majority the dorsiflexion axis was steeper and the plantar-flexion axis more nearly horizontal than is usual in the European ankle.



Text-fig. 3. Histograms showing that type I superior tibio-fibular joints are associated with steep dorsiflexion axes at the ankle; type III joints with nearly horizontal dorsiflexion axes.



Text-fig. 4. Graph showing that the degree of 'wedging' of the talus varies with the degree of inclination of the plantar-flexion axis.

RELATIONSHIP BETWEEN ANKLE AXIS, FORM OF TALUS AND MOBILITY OF FIBULA

An unexpected relationship was revealed when the inclinations of the ankle axis, as calculated in 152 specimens, were compared with the 'wedging' of the tali and the mobility of the fibulae.

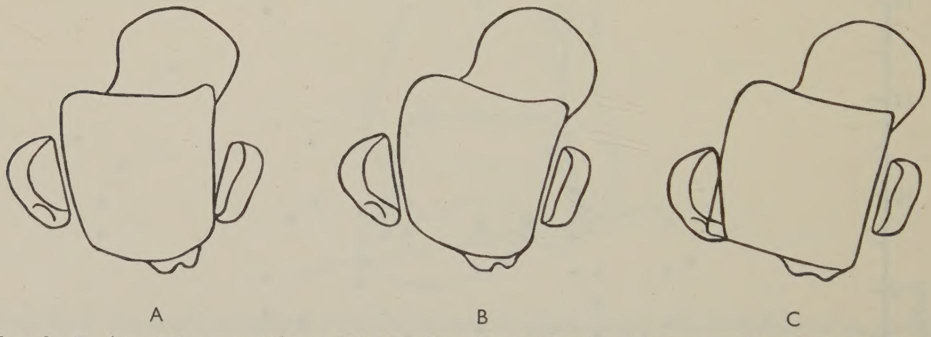
It was found that the mobility of the fibula was related to the steepness of the dorsiflexion axis. Where the superior tibio-fibular joint was large and nearly horizontal (type I), implying a freely mobile fibula, the inclination of the dorsiflexion axis was great; where it was small and steep (type III), implying a relatively immobile fibula, the inclination of the dorsiflexion axis was small (Text-fig. 3).

On the other hand, the degree of 'wedging' of the talus varied with the inclination of the plantar-flexion axis (Text-fig. 4); where this axis was almost horizontal it was found that the talus was parallel-sided (Pl. 1, fig. 8), while a steeply inclined plantar-flexion axis was always associated with a markedly 'wedged' talus (Pl. 1, fig. 7).

DISCUSSION

'Wedging' of the talus and the plantar-flexion axis

The downward and medial inclination of the plantar-flexion axis at the ankle produces a lateral deviation of the front of the talus relative to the tibia as plantar-flexion proceeds, which in turn leads to a separation anteriorly of the articular surfaces on the medial malleolus and the medial side of the talus (Text-fig. 5A).



Text-fig. 5. A, anterior gap at the medial side of the plantar-flexed left talus caused by the inclination of the plantar-flexion axis; B, gap closed by medial rotation of the talus about a vertical axis; C, medial rotation impossible if the talus is parallel-sided.

The lateral side of the talus, on the other hand, retains full contact with the adjoining facet on the fibular malleolus throughout plantar-flexion, since it is very nearly perpendicular to the plantar-flexion axis (Text-fig. 2). This relationship must follow from the general consideration that a rotating surface at right angles to its axis of rotation always maintains its original plane.

The non-alignment developing between the medial articular surface of the talus and the tibial malleolus during plantar-flexion can be neutralized by a medial rotation of the whole talus about a vertical axis.

Such a rotation would require the back of the lateral side of the talus to slope medially if it is to fit accurately against the lateral malleolus when rotation is complete (Text-fig. 5B, C). Examination of the lateral sides of the tali in this series reveals that many are shaped in this fashion. In some ankle joints it appears that this mechanism of medial rotation does not come into play until the final stages of plantar-flexion, for in these the 'wedging' is confined to the posterior one-third of the trochlear surface.

It is clear that this method of maintaining accurate alignment, and thus ensuring stability at the medial side of the talus during plantar-flexion, necessitates a narrowing of the trochlear surface of the talus from front to back. This 'wedging' is therefore greatest in those limbs where the plantar-flexion axis is steep, and minimal where the axis is so nearly horizontal that there is no tendency for the non-alignment to occur.

Rotation of the fibula during dorsiflexion

The medial side of the talus, facing slightly upwards as well as medially, is approximately at right angles to the dorsiflexion axis, which is inclined downwards and laterally (Text-fig. 1). It therefore retains its original plane throughout dorsiflexion, in accordance with the principle stated above.

On the other hand, the plane of the lateral side of the talus must change during dorsiflexion, since it makes an acute angle with the dorsiflexion axis. In order to maintain alignment with this side of the talus, the fibula must rotate laterally about its long axis during dorsiflexion. The actual range of this rotation, and consequently the type of superior tibio-fibular joint, will depend on the steepness of the dorsiflexion axis, becoming greater as the axis becomes steeper.

This rotation has been observed in the living as well as in a freshly amputated limb. Cineradiographic studies in suitable normal subjects indicate a slight rotational movement of the upper end of the fibula during dorsiflexion. This movement is difficult to demonstrate by this technique, however, and it has been observed only twice in a series of seven films. In two patients with wasting of the peroneal muscles, enabling the upper end of the shaft of the fibula to be palpated with ease, the lateral rotation has been readily observed.

The degree of fibular rotation was measured in one freshly amputated limb by means of metal indicator pins driven into the shafts of the tibia and fibula. During forced dorsiflexion, the fibula rotated laterally through 3 degrees relative to the tibia. Subsequent dissection showed that the superior tibio-fibular joint in this limb was large and flat.

The torsional effect upon the fibula produced by the inclination of the ankle axis obviously deserves consideration in the aetiology of fractures about the ankle occasioned by forced dorsiflexion strains.

Other movements at the ankle

It is unlikely that medial rotation of the talus and axial rotation of the fibula are confined exclusively to the plantar-flexion and dorsiflexion ranges respectively.

During plantar-flexion rotation of the fibula may occur in those cases where the lateral side of the talus is not truly perpendicular to the plantar-flexion axis, or where the 'wedging' of the talus is insufficient to maintain alignment at its lateral side as it rotates medially.

In some individuals, medial rotation of the talus, accompanied by medial rotation of the fibula, occurs when the dorsiflexed foot is adducted. This movement also can be observed by means of cineradiography. The report of the Committee on Artificial Limbs of the National Research Council (1947) stresses that such rotational movements of the talus at the ankle are more common than is generally appreciated.

It should be noted that the downwards and lateral inclination of the dorsiflexion axis produces a lateral deviation of the neck of the talus as dorsiflexion proceeds. Provided that no compensatory movements occur at the subtaloid joint, there is a tendency for the foot to deviate laterally as it is dorsiflexed. This deviation is accompanied by lateral rotation of the fibula as already discussed. Both these movements are relative to the tibia; if friction between the foot and the ground, engendered by weight bearing, is sufficient to prevent this lateral deviation, the tibia will rotate medially relative to the foot and the fibula during dorsiflexion.

SUMMARY

1. The trochlear surfaces of 152 cartilage-covered tali have been measured.

2. The medial and lateral profiles of the talus show differences in curvature which indicate that the axis of rotation at the ankle joint is a changing one. This axis is inclined downwards and laterally during dorsiflexion, and downwards and medially during plantar-flexion.

3. The expected correlation between the appearance of 'wedging' of the trochlear surface and the mobility of the fibula—judged by the nature of the superior tibio-fibular joint—has not been confirmed. However, correlation has been demonstrated between the steepness of the dorsiflexion axis and the mobility of the fibula, and between the steepness of the plantar-flexion axis and the degree of 'wedging' of the talus.

4. These relationships are discussed in terms of the possible mechanisms involved. It is suggested that mobility of the fibula and 'wedging' of the talus are necessary accompaniments of an ankle axis that is inclined during both dorsiflexion and plantar-flexion.

We are indebted to Prof. D. V. Davies for his constant encouragement and help, to Prof. H. A. Harris and Prof. M. F. Lucas Keene for generously providing material for study in their respective departments, and to Dr Russell J. Reynolds who kindly made several cineradiographic films for our benefit. Our thanks are due also to Mr J. E. Gibbs for technical assistance and to Mr A. L. Wooding for the photographic work.

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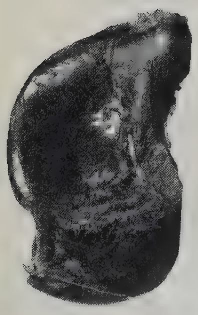
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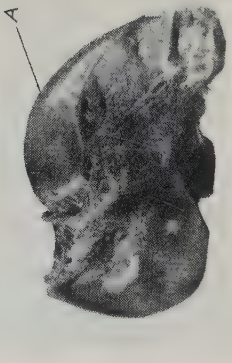

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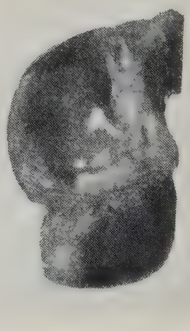
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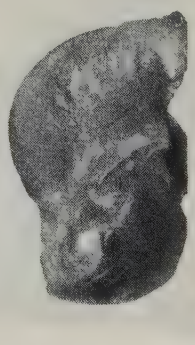
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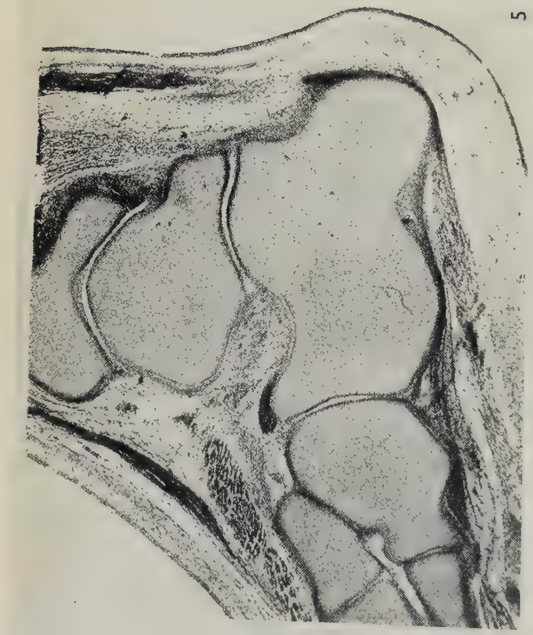
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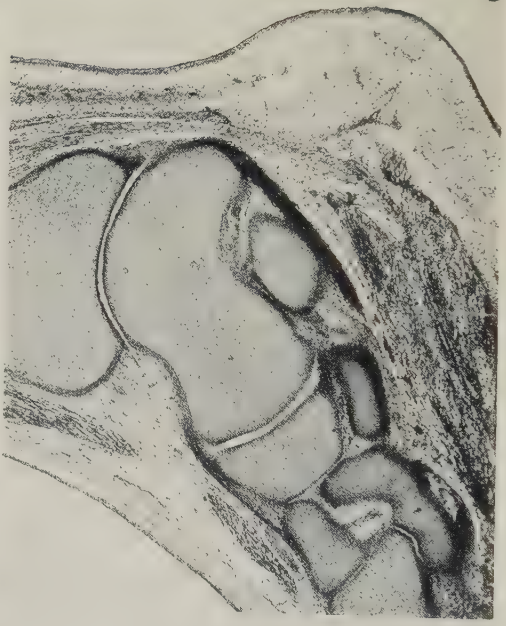
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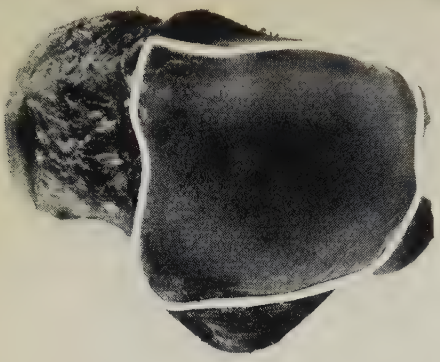
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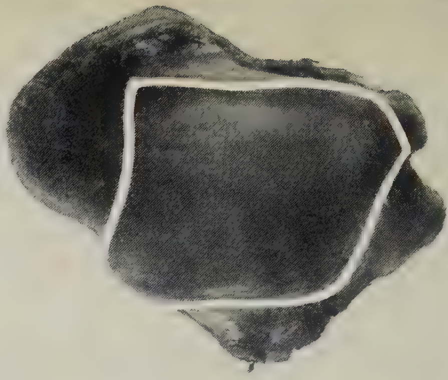
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BARNETT AND NAPIER—ROTATION AT THE ANKLE JOINT

EXPLANATION OF PLATE

Fig. 1. The lateral profile of a typical talus.

Fig. 2. The medial profile of the same talus (print reversed for ease of comparison). *A* indicates the highest point on the trochlear surface when the ankle is in the neutral position.

Fig. 3. The lateral profile of a rare type of talus having a fixed horizontal axis of rotation.

Fig. 4. The medial profile of the same talus (print reversed for ease of comparison).

Fig. 5. Section of foetal ankle region, near lateral edge of trochlear surface of talus. Eleven-week human embryo (crown-rump length, 50 mm.). ×30.

Fig. 6. Section taken near medial edge of trochlear surface of talus; same embryo. ×30.

Fig. 7. Trochlear surface of typical talus (shown in profile in figs. 1 and 2).

Fig. 8. Trochlear surface of the talus shown in profile in figs. 3 and 4.

A NEW METHOD FOR DEMONSTRATING MITOCHONDRIA

By J. J. PRITCHARD

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Some time ago it was found by the author that after Regaud fixation, Wilder's (1935) silver method for demonstrating reticular fibres in paraffin sections often showed what appeared to be mitochondria in the cytoplasm of many of the cells. This observation has been followed up, and a technique has been devised which regularly and specifically demonstrates mitochondria. Good preparations have a clarity and intensity of impregnation which surpasses that of the usual aniline-fuchsin and iron-haematoxylin techniques, and rivals that of the best preparations by the copper-chrome-haematoxylin method of Bensley. The last method, moreover, sometimes unaccountably fails to stain the mitochondria which alternative methods show to be present.

METHOD

Fixation

The classical Regaud method of fixation, including post-chromation, preserves mitochondria well, but if over-chromed other structures besides mitochondria may become silvered by the technique to be described, including nuclei, zymogen granules and collagen fibres. A more satisfactory fixative was found to be 10 % formol-saline for 24 hr., followed by 48 hr. in 3 % potassium dichromate. Helly's fluid preserves mitochondria less well, as there is a tendency for filamentous forms to become fragmented into short rods and granules. However, after Helly fixation, a Golgi reticulum can often be seen in addition to the mitochondria, and this simultaneous impregnation might be useful for some purposes. Strong Flemming's solution, without acetic acid, was also successfully employed.

Preparation of sections

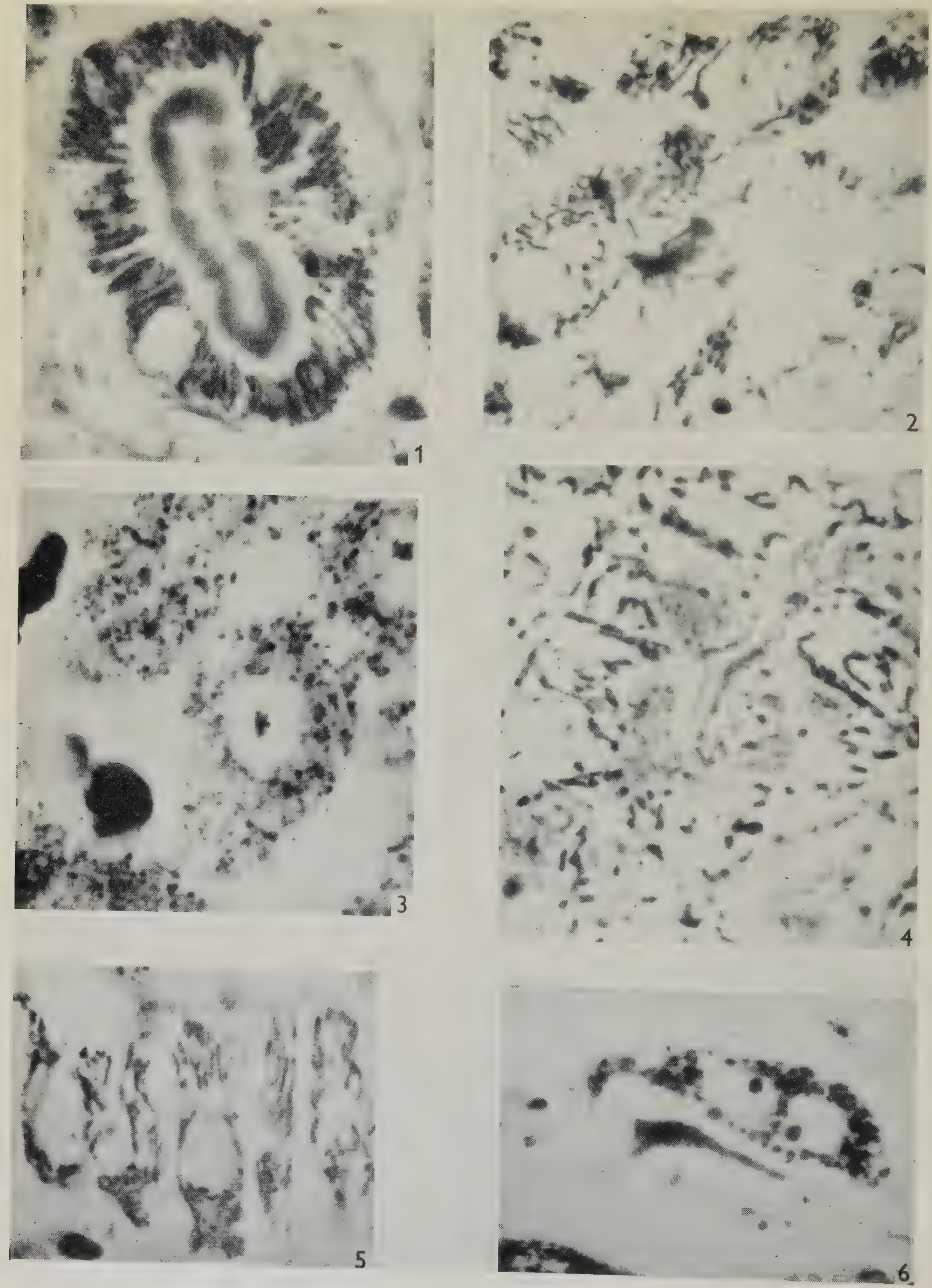
After washing in running tap water overnight, dehydration, clearing and impregnation with paraffin in the usual way, sections are cut as thin as possible (2–5 μ) and mounted with the aid of glycerine-albumin, avoiding excessive heat in the flattening and drying processes.

Treatment of sections

(1) After removal of paraffin, sections are brought down to distilled water.

(2) Without any other preliminary treatment sections are placed, with gentle agitation, for 20–30 sec. in the silver bath. This consists of Wilder's (1935) silver diammino hydroxide ($Ag[NH_3]_2OH$) solution diluted with an equal volume of distilled water with the addition of 2 drops of 8 % NH_4OH per 50 c.c. of the mixture.

(3) Excess silver solution is drained quickly from the slide which is plunged, with agitation, into a jar containing 0·1 % commercial formalin in distilled water. Reduction takes place within a few seconds and the slide should be removed after 10 sec. Fresh formalin solution should be used for each section.



PRITCHARD—A NEW METHOD FOR DEMONSTRATING MITOCHONDRIA

(4) The slide is then washed in distilled water and examined under the staining microscope. In successful preparations the mitochondria are specifically blackened and show very distinctly. A yellowish or brownish staining of nuclei and other structures is often encountered, but this can be removed with 2 % aqueous potassium ferricyanide, the differentiation being controlled under the microscope.

(5) After thorough washing in distilled water preparations may be counterstained with saffranin, Ponceau fuchsin, haematoxylin and eosin, or methylene blue.

Comment

Examples of preparations made by this method are illustrated in Pl. 1, figs. 1–6. A series of similar sections stained with aniline-fuchsin-methyl green, iron-haematoxylin, Bensley's copper-chrome-haematoxylin and by the above silver-reduction method demonstrated the superior precision and staining intensity of the last-named technique. The method fails, as do all staining methods for mitochondria, at the centre of large blocks of tissue, where the mitochondria have disintegrated before the fixative has reached them. Too long a period of post-chromation or in the silver bath, too strong a formalin solution, insufficient or too much ammonia in the silver bath, mechanical damage to tissues and sections, and excessive heat used in flattening and drying sections are all disadvantageous. The best preparations were made soon after drying the sections, although good results have been obtained with mounted sections stored for several months.

SUMMARY

A simple and rapid silver-reduction method for demonstrating mitochondria in paraffin sections is described.

REFERENCE

Wilder, H. C. (1935). An improved technique for silver impregnation of reticulum fibers. *Amer. J. Path.* **11**, 817–819.

EXPLANATION OF PLATE

Fig. 1. Adult rat: proximal convoluted tubule of kidney, showing basally situated mitochondria. × 1800.

Fig. 2. Foetal rat, 16 days: a group of osteoblasts from the deep zone of the periosteum of the mandible, showing filamentous mitochondria. × 1800.

Fig. 3. Adult rat: liver cells, showing an abundance of granular and short rod-like mitochondria. × 1800.

Fig. 4. Adult rat: pancreatic acinus, showing long stout mitochondria at the basal ends of the cells. Zymogen granules near the lumen are shown indistinctly. × 1800.

Fig. 5. Adult rat: epithelium of jejunum, showing filamentous mitochondria between the nucleus and the free border, and granular and short rod-like mitochondria at the basal end of the cell. × 1800.

Fig. 6. Foetal rat: binucleated osteoclast from the mandible, showing numerous granular and short rod-like mitochondria. × 1800.

THE DEVELOPMENT OF HALVED TOOTH GERMS. A STUDY IN EXPERIMENTAL EMBRYOLOGY

By SHIRLEY GLASSTONE
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THE DEVELOPMENT OF HALVED TOOTH GERMS IN TISSUE CULTURE

In previous work (Glasstone, 1938) it was shown that molar tooth germs of the rabbit when cultivated *in vitro* displayed remarkable powers of development. Molar tooth germs explanted before cusps had appeared developed these structures during cultivation. This showed that the factors responsible for cusp development are intrinsic in the tooth germs themselves and are independent of external mechanical conditions in the developing jaw. The nature of these intrinsic factors is not understood.

The object of the present experiments was to see whether, in tooth germs where cusps have not yet formed, the fate of the cusp-forming regions is already rigidly determined, or whether any regulative changes could occur when the tooth germs were divided into two.

In the present experiments tooth germs were halved at various periods relative to the first appearance of cusps, and the halves were then cultivated separately.

MATERIALS AND METHODS

The experimental material consisted of the first and second temporary lower molars of the rabbit foetus, in which cusps form during the 21st day. In these experiments embryos of 20, 21 and 22 days were used. To determine the stage of development of the tooth germs at explantation, the corresponding molars were removed from each half of the lower jaw; of each pair one was divided and the halves cultivated, and the other was also cut in half before being fixed as a control (Pl. 1, fig. 1, Pl. 2, fig. 1).

The tooth germ removed for cultivation was cut into two in a bucco-lingual direction. (The usage of such topographical terms is defined in Glasstone, 1938). The two fragments were not necessarily of equal size. It was not found possible to divide the tooth germs along any other plane without seriously damaging the tissue.

The halved molars were grown by the hanging-drop method on $1\frac{1}{4}$ in. square cover-slips. The two halves of each tooth germ were cultivated on one cover-slip, but were as far as possible kept apart from each other, though in some experiments the two fragments came very close and sometimes became joined by their outgrowths. The culture medium consisted of three drops of plasma to one drop of embryo extract of an 11-day-old chick embryo; the extract was made with Pannett and Compton's saline containing 1 % glucose; the cultures were incubated at 37° C. Some explants were transferred to fresh medium after 4 days, but most were left undisturbed. The divided tooth germs were fixed at intervals of 1–7 days and sectioned. They were removed from the clot and fixed in 3 % acetic Zenker solution for 30 min., then washed, dehydrated, cleared in methyl benzoate and embedded in paraffin wax. The controls were similarly treated. All sections were stained with iron-haematoxylin and chromotrop. Wax reconstructions of the dentine papillae of the cultures were made by the method previously described (Glasstone, 1938).

OBSERVATIONS. DEVELOPMENT OF CULTIVATED HALVED TOOTH GERMS

A. *Twenty-day-old embryos*

At 20 days there is no sign of cusp formation in the oval tooth germs. The explants consisted of halved tooth germs which had been dissected from the jaw. The rate at which the halves developed depended on their size. Each part suffered some damage when cut, but all except the smallest fragments were able to recover; sometimes even these remained healthy but they did not differentiate, and occasionally became necrotic. In particular, the enamel organ, which is a membranous structure, was damaged in dissection and the extent to which it recovered was variable. On its regeneration depended the formation of cusps within the papilla, for without it cusps cannot form (Glasstone, 1936). When the fragments were of approximately equal size, and the enamel organ regenerated, each fragment developed as if it were a whole tooth germ. The explant became rounded and the enamel organ grew round the cut surface of the dentine papilla, which proceeded to form cusps. Occasionally, cusps did not form, although the enamel organ regenerated.

Previously (Glasstone, 1938), it was shown that the cusp pattern of the explanted whole tooth was similar to the normal although much smaller (Pl. 2, figs. 2*a*, *b*). The same was true of the halved tooth germ which, however, was still smaller than the normal structure.

Where cusps formed they still followed the normal pattern. In this, a deep fissure separates a small distal cusp from a larger proximal group of cusps in which central, lingual and buccal cusps can be distinguished (Pl. 2, figs. 3, 4). In the whole tooth germs in culture, additional cusps in the proximal group were sometimes found (Glasstone, 1938) and this also happened in some of the halved tooth germs. Occasionally the subdivision of the whole proximal group was imperfect (Pl. 2, fig. 4). Histological development was the same as shown previously (Pl. 1, fig. 2) (Glasstone, 1938). Odontoblasts and dentine were observed, though in some of the cultures the odontoblasts become degenerate and in them occasional pycnotic nuclei were seen. In all the cultures in which odontoblasts and dentine formed, ameloblasts were present.

B. *Twenty-one-day-old embryos*

In 21-day-old tooth germs the early rudiment of the fissure between proximal and distal cusps is first observed. The explants of halved tooth germs from 21-day-old embryos grew in much the same way as those 1 day younger and developed as a whole tooth germ (see Table 1), except that the proportion of instances was greater in which the cusps in each half gradually approximated to the normal pattern. Possibly this is due to the more ready recovery of the larger explant. Histological development was the same as in the explants of 20-day-old embryos.

Table 1. *Number of tooth germs exampled*

Age of foetus in days			
20	21	22	
8	16	—	Complete regulation to two tooth germs
19	7	1	Complete regulation in one tooth germ, 2nd half degenerate
4	6	1	Complete regulation in one tooth germ, 2nd half showing partial development
11	1	1	Complete regulation in one tooth germ, 2nd half no development
8	—	6	No development in two halves
—	—	8	No regulation, each half formed its own cusps
—	—	3	One half formed its own cusps, 2nd half degenerated
50	30	20	Total

C. *Twenty-two-day-old embryos*

During the 22nd day the fissure between the proximal and distal cusps becomes much deeper. When the tooth germs were divided and cultivated, neither half formed a complete tooth (see Table 1). Each half formed its own cusps, and in some instances additional cusps were seen in the proximal group as in some cultivated whole tooth germs.

DISCUSSION

Experimental embryology has shown that before a given stage of development the fate of certain tissues can be experimentally modified and that cells which normally would form one type of structure can be made to follow a different path in development. After this point, when the tissue is said to be determined, this plasticity of behaviour is lost. Determination usually precedes any visible change in the tissue.

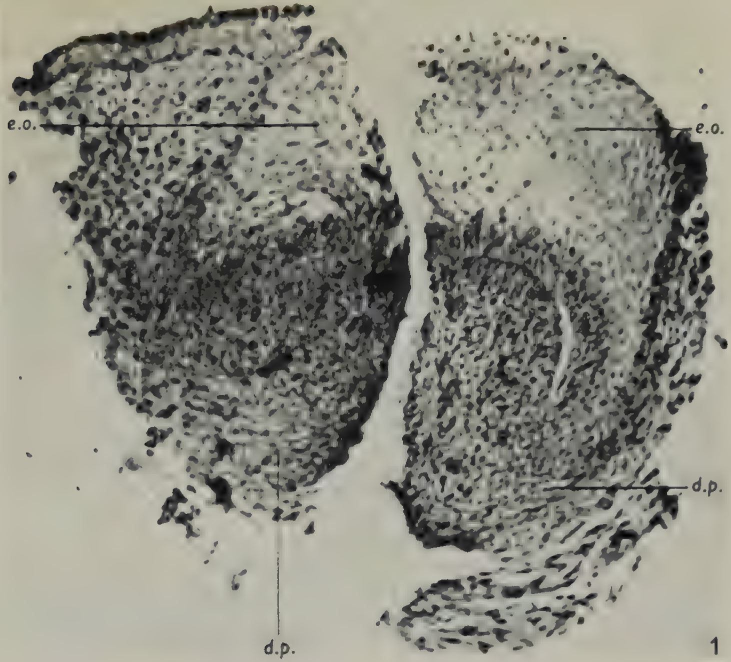
The present study has demonstrated that the cusp-forming regions are not morphologically determined in the tooth germs at early stages of development, since half tooth germs often form complete tooth rudiments when cultivated *in vitro*. These half rudiments, although smaller than explants of whole tooth germs, follow the same developmental pattern even to the extent of sometimes forming additional cusps in the proximal group. Odontoblasts, dentine and ameloblasts differentiate in many cultures.

This capacity for morphological regulation in explanted halves persists up to the 22nd day of foetal life, that is until just before odontoblasts and dentine have begun to form, but after this stage regulative changes can no longer take place in cultures.

The author wishes to express her thanks to Dr H. B. Fell for her help; and also to the Medical Research Council for a personal grant and for defraying the expenses of the investigation.

SUMMARY

1. In the development of molar teeth in the rabbit, cusps are first discernible at 21 days.
2. Halved molars were explanted from embryos of 20–22 days, and cultivated for periods up to 7 days.
3. At 20–21 days, the halved germs developed their normal pattern of cusps.
4. At 22 days, when odontoblasts and dentine appear, this capacity for regulative development is largely lost. The normal cusp pattern has then been determined.
5. As in explants of whole tooth germs, odontoblasts differentiate, dentine is formed, and the cells of the internal enamel epithelium differentiate into ameloblasts.



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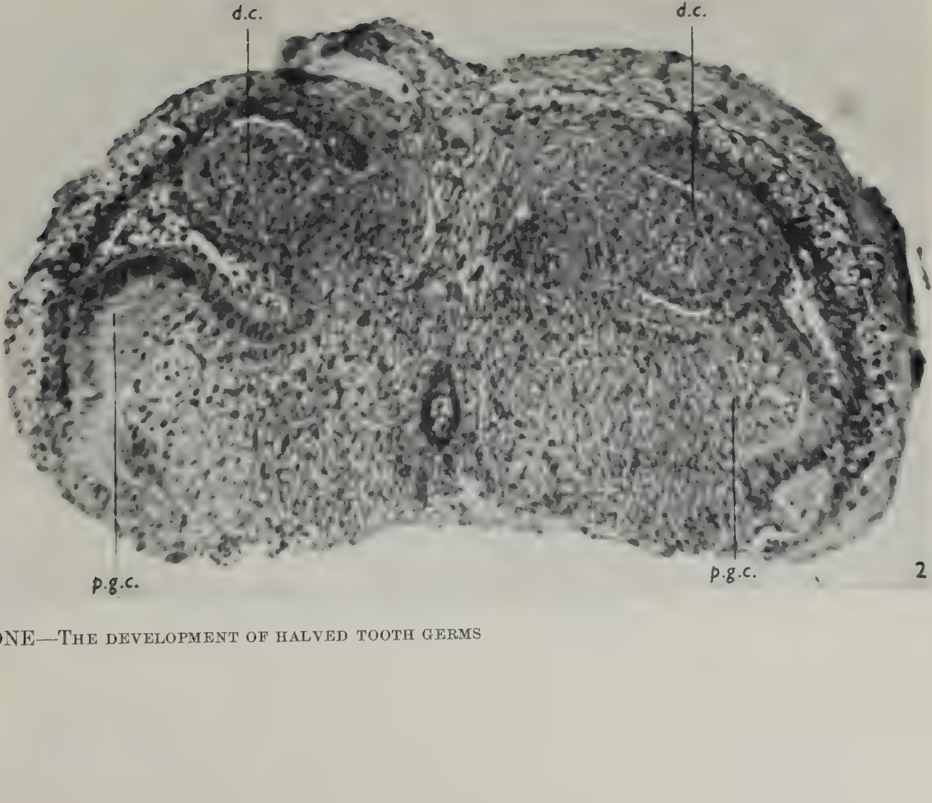

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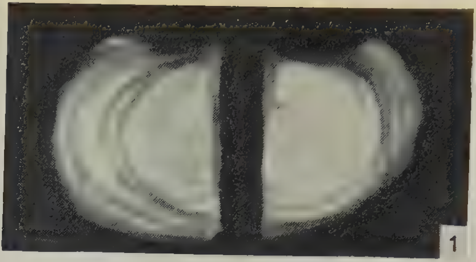
GLASSTONE—THE DEVELOPMENT OF HALVED TOOTH GERMS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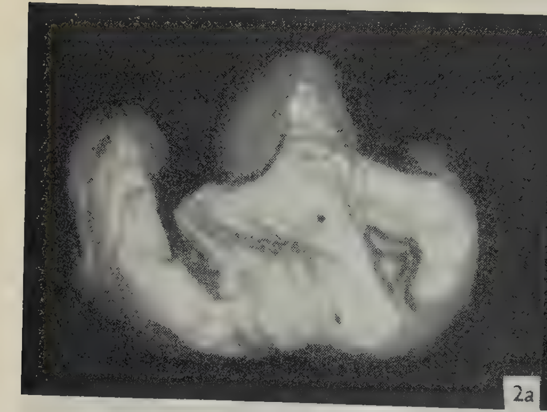
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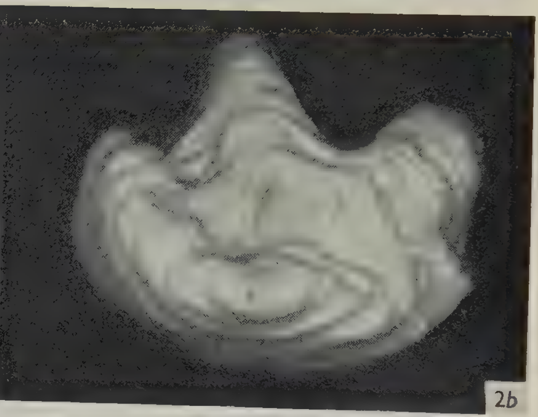
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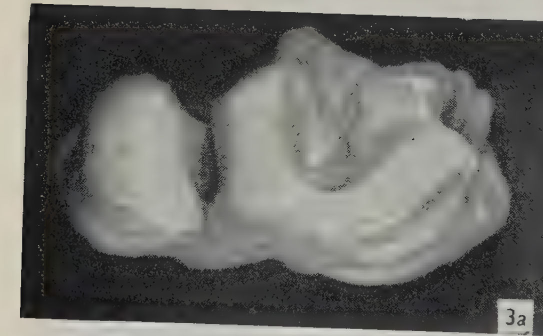
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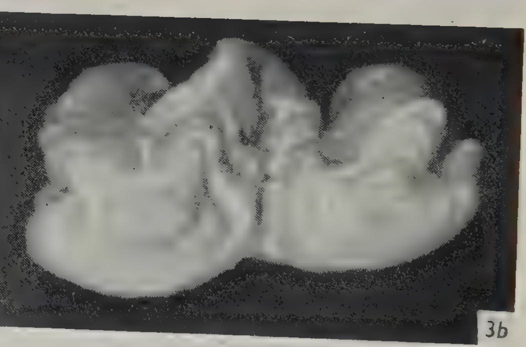
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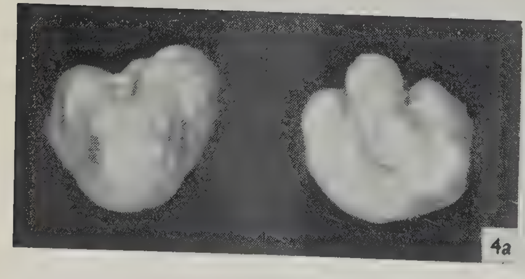
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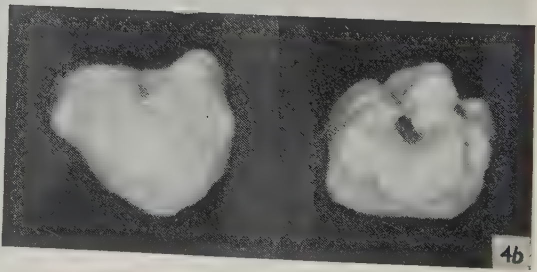
4b

GLASSTONE—The development of halved tooth germs

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EXPLANATION OF PLATES

PLATE 1. Twenty-day-old foetal molar

Fig. 1. Longitudinal section halved as for experiment. The cusps have not yet formed. (×200.)

Fig. 2. Longitudinal section of halved tooth germ after cultivation for 5 days. Each half has formed a complete set of cusps. Their outgrowths have fused. (×200.)

PLATE 2. Photographs of wax reconstructions of dentine papillae of 20-day-old foetal molars (×80.)

Fig. 1. Lingual view before cultivation. Halved as for experiments. Cusps have not yet formed.

Fig. 2. Whole tooth germ after cultivation for 5 days. (*a*) Buccal view—single distal cusp on left—large proximal group on right. (*b*) Lingual view—large proximal group on left, single distal cusp on right.

Fig. 3. Halved tooth germs after cultivation for 5 days. Each half has formed a complete set of cusps. The distal cusp of each is apposed. Their outgrowths have fused. (*a*) Buccal view. (*b*) Lingual view.

Fig. 4. Halved tooth germs after cultivation for 5 days. One half on left in both (*a*) and (*b*) is imperfect. In the other, regulation is complete. (*a*) Buccal view—the proximal group is on the right in the left tooth germ and on the left in the right tooth germ. (*b*) Lingual view—the proximal group is on the left in the left tooth germ and on the right in the right tooth germ.

List of abbreviations

d.c.	distal cusp	*e.o.*	enamel organ
d.p.	dentine papilla	*p.g.c.*	proximal group of cusps

ON THE RELATIONS OF THE HUMAN VOMER TO THE ANTERIOR PARASEPTAL CARTILAGES

By F. C. ELOFF
Department of Zoology, University of Pretoria

Since Zuckerkandl's paper dealing with the ossification of the mammalian vomer appeared in 1909, numerous statements on the relationship between this bone and the nasal capsule have been made. The most important contributions, so far as the present discussion is concerned, are those of Fuchs (1909) and Fawcett (1911).

Fuchs's observations (which are substantially in agreement with those of Zuckerkandl) can be summarized as follows: in 50–60 mm. cat embryos a portion of the caudal half of the vomer becomes intimately related with the medial side of the paraseptal cartilages and, farther backwards, with the lamina transversalis posterior. In 90–100 mm. cat embryos the vomer has expanded considerably and has come to surround the paraseptal cartilage completely. The bone actually invades the cartilage, which ossifies as cartilage bone. This replacement ossification becomes incorporated into the definitive vomer. A similar addition to the vomer takes place farther backwards at the point where this bone is fused to the lamina transversalis posterior.

This tendency of the vomer to enlarge at the expense of the tissue of the paraseptal cartilages and the posterior transverse laminae led Fuchs to conclude that the vomer is ontogenetically a mixed bone, including both membrane and cartilage bone components.

Fawcett's contribution (1911), based on observations made on a number of human embryos, consists mainly of a corroboration of Fuchs's and Zuckerkandl's remarks on the ossification of the vomer in the cat. He points out that the human vomer, although of membranous origin, becomes added to by ossification of the hinder end of the anterior paraseptal cartilages in the 100 mm. stage. From this ossified posterior end a plate of membrane bone projects downwards and in the 150 mm. stage fuses with the outer edge of the plate connecting the two limbs of the Y-shaped vomer. Referring to fig. 19 of his paper, Fawcett states that this is quite clearly the condition observed in the cat by Fuchs and Zuckerkandl. On the basis of these observations Fawcett reaches some conclusions regarding the phylogeny of the mammalian vomer and its supposed original relations to the anterior paraseptal cartilages.

Fawcett's statement, however, does not seem to be consistent with his observations. In Fuchs's figures it is very clearly shown that the vomer has actually invaded the anterior paraseptal cartilage and is in direct contact with it, no periosteal or perichondrial layers intervening. In Fawcett's fig. 19 the vomer and the ossification derived from the anterior paraseptal cartilage are, though admittedly very close to each other, clearly separated by both their periostea, and there is no question of fusion between the two. This condition does not correspond with that described by Fuchs and Zuckerkandl in the cat, as Fawcett maintained.

In 1931 Augier published the results of his investigations on 300 human embryos treated by the Schultz and Spalteholz methods. His main conclusions are:

(1) The ossification of the anterior paraseptal cartilage does not develop by extension from the vomer. It arises independently and forms a paraseptal ossicle.

(2) The paraseptal ossicle ends by fusing with the vomer and becomes merged into it during the last months of intra-uterine life or after birth.

These conclusions are quite clearly not compatible with Fawcett's observations. Unfortunately Augier, relying solely on macroscopic preparations of cleared material, was not in a position to make minute and detailed observations. This makes a review of his and Fawcett's conclusions highly desirable.

Through the kindness of Prof. J. D. Boyd I have had access to his extensive collection of human material and was able to make the following observations on a series of human embryos ranging in length from 92 to 265 mm.

Although Augier maintains that the paraseptal cartilages commence to ossify in the 60 mm. stage, in neither the 92 mm. nor the 99 mm. stages examined could any signs of ossification be detected. The vomer is well separated from the paraseptal cartilage in both these stages; there is no evidence that it becomes added to by ossification of the hinder end of the anterior paraseptal cartilages as Fawcett observed it to do in the 100 mm. stage. These two stages are important though, in that one is able to establish the very close relations that exist, not between vomer and paraseptal cartilages, but between the latter and the premaxilla. This is noticeable in both stages, but especially so in the 99 mm. stage, where the perichondrium of the paraseptal cartilages extends uninterruptedly into the membrane of condensed mesenchyme that surrounds the palatal (prevomerine) process of the premaxilla (Pl. 1, fig. A). At these early stages, therefore, the paraseptal cartilages are more closely associated with the premaxilla than with the vomer.

In the 108 mm. stage the anterior paraseptal cartilage does not yet show any sign of ossification, but immediately behind its posterior end there is a tiny independent membranous ossification. The anterior margin of this ossification slightly overlaps the posterior extremity of the paraseptal cartilage, but is quite distinct from it (Pl. 1, fig. B). This membranous ossification is undoubtedly the element which Augier calls paraseptal ossicle and which he also found to be of independent origin. The nature of his material, however, did not allow him to observe the true relations between this ossicle and the anterior paraseptal cartilage in its further development.

Two specimens of 145 and 150 mm., respectively, show an essentially similar condition in regard to the relations between vomer and the anterior paraseptal cartilage and its ossicle. Attached to the posterior end of each anterior paraseptal cartilage there is a large plate of membrane bone which descends on each side of the vomer (Pl. 1, fig. C). Although the two elements are very close to each other they are clearly distinct and each retains its separate identity. This membranous plate, which ends as a free element on the outside of the vomer, is quite clearly the independent ossification observed in the 108 mm. stage. It has now invaded the posterior end of the anterior paraseptal cartilage which is seen to be undergoing perichondrial as well as endochondrial ossification.

In Fawcett's description of the 150 mm. stage he maintains that the posterior end of the paraseptal cartilage is undergoing this type of ossification as a result of its invasion by the vomer. It is possible that Fawcett erroneously regarded the paraseptal ossicle described by Augier and the present author as part of the vomer.

In the 200 mm. stage the posterior ossification of the anterior paraseptal cartilage seems to be relatively smaller than in the previously described stage. The vomer has invaded the membrane by which the anterior paraseptal cartilage and its posterior ossification seem to be connected to the nasal septum, but it does not enter into closer association with the paraseptal ossification than in the previous stage.

In a 265 mm. specimen two curious variations occurred. The paraseptal ossicle was present on the right side only and was not fused to the anterior paraseptal cartilage (Pl. 1, fig. D).

While it is clear that this region of the developing skull is subject to considerable individual variation, the following conclusions can be drawn from the material examined:

(1) Augier's statements regarding the independent anlage of the paraseptal ossicle are confirmed.

(2) The paraseptal ossicle invades the posterior end of the anterior paraseptal cartilage and enlarges at the expense of the tissue of the cartilage.

(3) Up to the 265 mm. stage the vomer does not fuse with either anterior paraseptal cartilage or its posterior ossification.

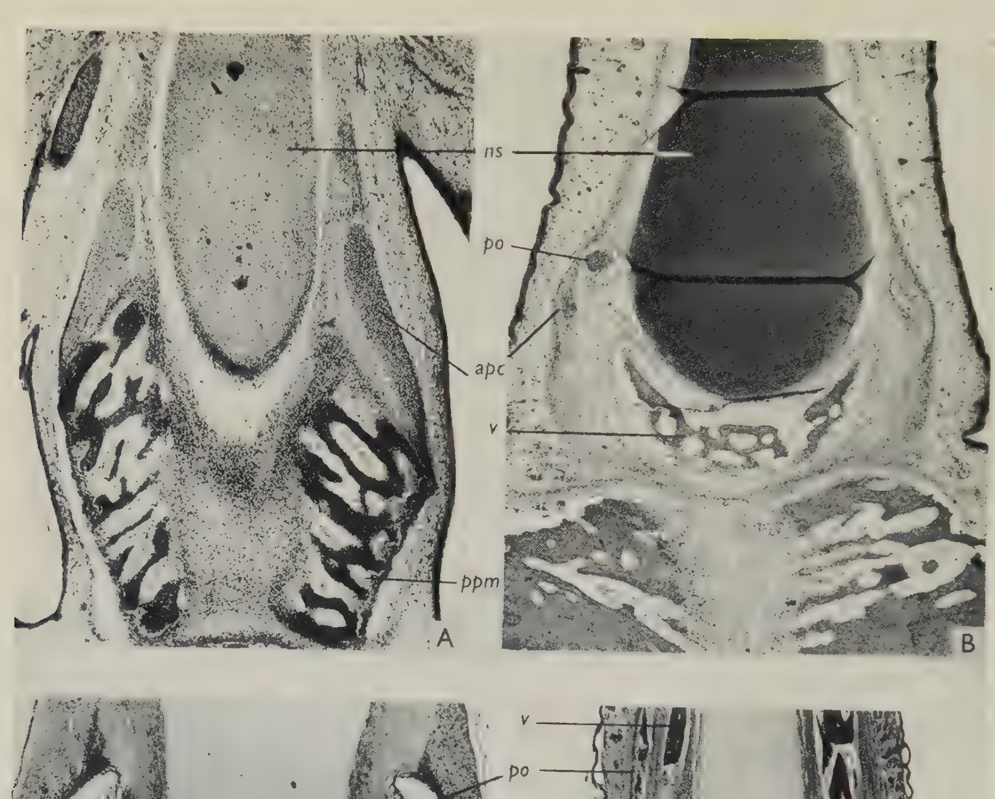
(4) In the early stages examined the anterior paraseptal cartilage is more intimately associated with the premaxilla than with the vomer.

(5) The supposed relationship between vomer and anterior paraseptal cartilage, as maintained by Fawcett, could not be confirmed. Augier's statement that the paraseptal ossicle becomes merged with the vomer during the last months of intrauterine life or after birth does not indicate any significant relationship between these two elements, as it is simply a stage in the normal course of development of two membrane bones of the skull. The premaxilla and maxilla, for instance, fuse with each other at the 27 mm. stage.

SUMMARY

A number of human embryos, ranging in length from 92 to 265 mm., were examined to investigate the true relations between vomer and anterior paraseptal cartilages at various stages of development. An independent paraseptal ossicle made its appearance in a 108 mm. specimen behind the anterior paraseptal cartilage. In the next stage examined (145 mm.) this ossicle has invaded the anterior paraseptal cartilage, and develops at the expense of the latter. At no stage was any fusion between vomer and anterior paraseptal cartilage or between vomer and paraseptal ossicle observed.

I would like to express my indebtedness to Prof. J. D. Boyd of the Anatomy Department, London Hospital Medical College, for access to the material and for providing the necessary facilities. My thanks are also due to Dr A. d'A. Bellairs for much helpful advice and to Mr R. Cox for technical assistance.



ELOFF—Relations of human vomer to paraseptal cartilages

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EXPLANATION OF PLATE

Fig. A. Horizontal section, 99 mm. embryo; × 32. B. Coronal section, 108 mm. embryo; × 26. C. Coronal section, 150 mm. embryo; × 33. D. Horizontal section, 265 mm. embryo; × 13. *apc*, anterior paraseptal cartilage; *ns*, nasal septum; *po*, paraseptal ossicle; *pm*, premaxillary bone; *ppm*, prevomerine process of premaxilla; *v*, vomer.

THE PROJECTION OF THE PULVINAR TO THE TEMPORAL LOBE

By D. A. SIMPSON
Department of Anatomy, University of Oxford

Kao Liang Chow (1950) has recently presented a study of the cortical projection areas of the pulvinar in *Macaca mulatta*. In addition to a widespread representation in the occipital and parietal lobes and the posterior extremity of the temporal lobe, he describes a remarkably localized (and apparently isolated) projection from the posterior half of the nucleus pulvinaris medialis to the lateral surface of the temporal pole. This last connexion had not been observed by earlier workers (Le Gros Clark & Northfield, 1937; Walker 1938). On the other hand, it is in accord with the observations of Bucy & Klüver (1940), who noticed degeneration in the ventral part of the inferior nucleus of the pulvinar, and in the posterior parts of the medial and lateral nuclei, following bilateral temporal lobectomy. The caudal limit of their lobectomy, it may be noted, was at the level of the inferior end of the central sulcus. Bailey, Bonin & McCulloch (1950), who refer to the work of Bucy & Klüver, suggest the third and basal temporal convolution as a possible thalamic projection area, but present no experimental evidence for this supposition.

Since earlier workers had found no specific projection to the temporal pole, Chow's observation that one of the nuclear elements of the pulvinar projects to this region is somewhat unexpected. It therefore seemed desirable to seek experimental confirmation of his results. At the same time, the opportunity has been taken of examining and reporting on a human brain with a circumscribed cortical lesion in a corresponding area of the temporal pole.

NOMENCLATURE

The pulvinar nuclei have suffered under a diversity of names. On the whole, the system of Aronson & Papez (1934) is most convenient because of its simplicity, and for this reason it has been adopted here. The different terminologies which have been applied to the nuclear elements of the macaque pulvinar are as shown in Table 1.

Table 1

Aronson & Papez (1934)	Friedemann (1912)	Grünthal (1934)	Walker (1938)
P1	Pδ	Griseum Intergeniculatum	N. pulvinaris inferior
P2	Pμ dorsally, Pη ventrally	P1 and P2	N. pulvinaris lateralis
P3	Pβ; the dorsal superficial zone is distinguished as Pla.		N. pulvinaris medialis
P4	Pα	—	(Not separately named)
(Nucleus of the optic tract)	(Nucleus limitans)	P3	(N. limitans, etc.)

Descriptions of the human pulvinar (Toncray & Krieg, 1946; Kuhlenbeck, 1951, and others) show a nuclear configuration resembling that of the macaque, though the increased development of this part of the thalamus has led to some alteration in the proportions and topographical relationships of the nuclear components.

EXPERIMENTAL RESULTS

Experiment 1. *Macaca mulatta* (MP 95)

The left superior temporal gyrus was exposed in its anterior extent, and the vessels in the parallel sulcus coagulated. A lesion was made with suction in this gyrus.

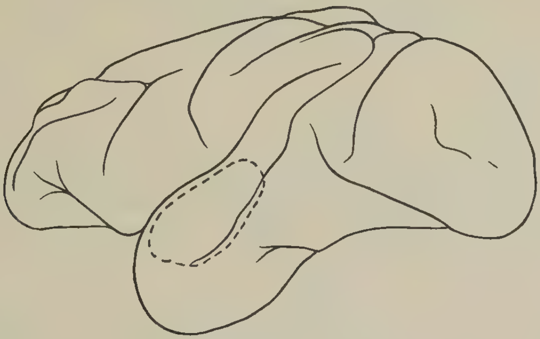

Fig. 1*a*.

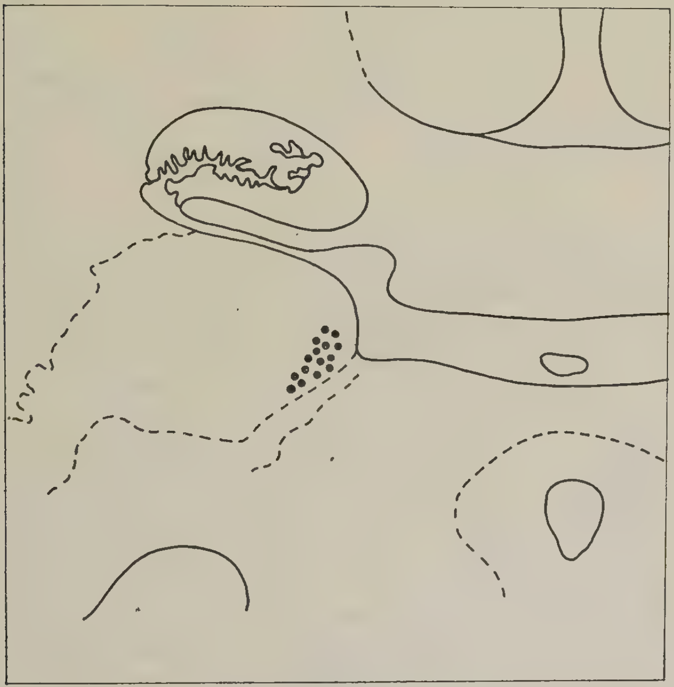

Fig. 1*b*.

Text-fig. 1. (*a*) The cortical lesion in Exp. 1. (*b*) Coronal section through the pulvinar region in Exp. 1, drawn with a projection apparatus. In this and the following figures, the areas affected by retrograde degeneration are indicated by dots.

Above and behind, the ablation was limited to the cortex, but below and in front it was made to extend into the subjacent white matter in order to interrupt fibres passing to the tip of the temporal pole. After 29 days the monkey was killed with nembutal and perfused with 4 % formol saline. The brain was cut coronally and blocks including the pulvinar region were embedded in paraffin, sectioned in the coronal plane at 15 μ, and stained with Borrel's methylene blue. The lesion proved

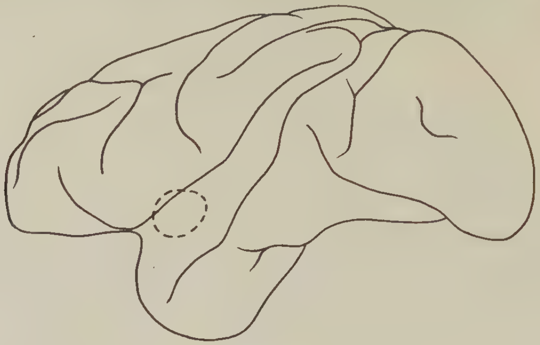

Fig. 2*a*.

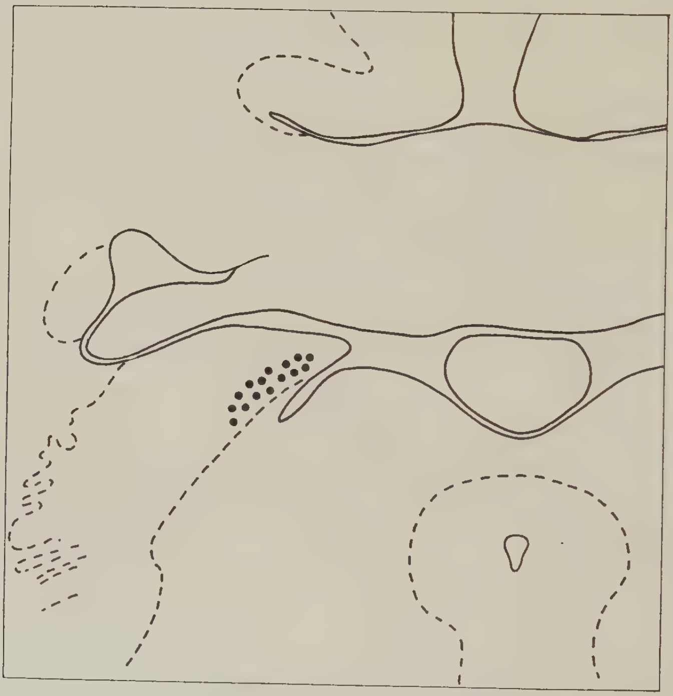

Fig. 2*b*.

Text-fig. 2. (*a*) The cortical lesion in Exp. 2. (*b*) Coronal section through the pulvinar in Exp. 2, drawn with a projection apparatus.

to be 21 mm. long and 9 mm. wide superficially (Text-fig. 1*a*). It does not encroach on the buried margins of the Sylvian fissure, but the cortex in the depths of the superior temporal sulcus shows softening adjacent to the lesion. Histological examination makes it probable that the lesion involves area 22 (TA of von Economo & von Bonin) with a slight encroachment on area 21 (TE).

The left pulvinar shows an area of partial cell loss confined to the posterior and ventral part of its medial nucleus P3 (Text-fig. 1*b*; Pl. 1, fig. 1*a*). This area extends rostrally from the caudal limit of the line of fusion between pulvinar and midbrain to the caudal margin of the posterior commissure. The area of cell loss is not sharply defined; it involves only a small, infero-medial, part of the large nucleus P3. The cell loss is accompanied by gliosis. The element P4, which Walker (1938) refers to as an unnamed cell group without cortical connexions, is intact, and so is the nucleus limitans.

Experiment 2. *Cercopithecus sabaeus* (MP 97)

As in the previous case, the left superior temporal gyrus was exposed, but a smaller cortical ablation was made with suction. After 29 days the monkey was killed and the brain fixed in 70 % alcohol. Large blocks were embedded in nitrocellulose and cut coronally at 25 μ. Every seventh section was stained with Borrel's methylene blue.

The lesion, which is circular and approximately 7 mm. in diameter, involves the dorsal and ventral lips of the Sylvian fissure (Text-fig. 2*a*). It was examined histologically; from its position, and by comparison with the cyto-architecture of the opposite side, it appears to lie in area 22 (TA) with a slight encroachment on to the frontal lobe.

Partial retrograde degeneration with a definite gliosis is seen in the pulvinar element P3, as in the preceding case, and no other nucleus appears to be affected (Text-fig. 2*b*). The area of cell loss is apparently smaller, however, in its antero-posterior extent.

Experiment 3. *Macaca mulatta* (M 51*a*)

In connexion with a study of the projection of the centre median nucleus made several years ago (Le Gros Clark & Russell, 1939), this monkey was subjected to a devascularization of the Island of Reil. The blood vessels all along the lips of the Sylvian sulcus were coagulated, as well as the branches of the middle cerebral artery crossing the limen insulae. In addition to the insular cortex, the superior temporal gyrus was devascularized by this procedure. Thirty-one days later, the animal was killed with ether and the brain removed. The following description is taken from the original paper. 'In its superficial extent, the lesion was confined to the immediate margin of the Sylvian sulcus and measured 29 mm. in length. It was limited caudo-ventrally by the parallel sulcus' (see Text-fig. 3*a*, which is redrawn from fig. 1 of the original report). Histologically, the lesion is stated to be 'limited to the cortex and the immediately subjacent white matter. The whole of the insular cortex had undergone complete necrosis, as well as the submerged cortex forming the lips of the Sylvian sulcus.'

'Serial transverse sections were taken through the thalamus and stained with methylene blue. These show well-defined areas of cell atrophy and accompanying gliosis in the following places—the ventral margin of the dorso-medial nucleus, the ventro-medial region of the ventral nuclear mass, the medial part of the main element of the pulvinar (nucleus Pβ of Friedemann) extending back as far as the level where the pulvinar forms a freely projecting pole, and the medial geniculate body.' Re-examination of the excellent sections prepared in this study confirms the description given above. Owing to the fact that the plane of section is somewhat oblique, some difficulty was experienced in defining the area of cell loss in terms of the nuclear elements of the pulvinar. However, the estimated extent of the

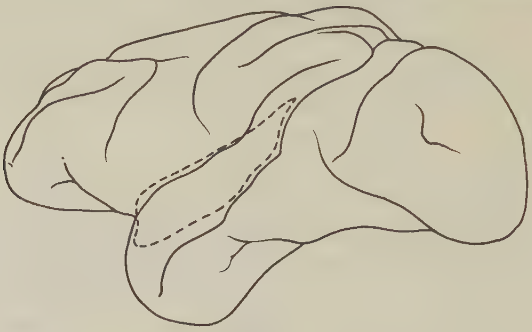

Fig. 3*a*.

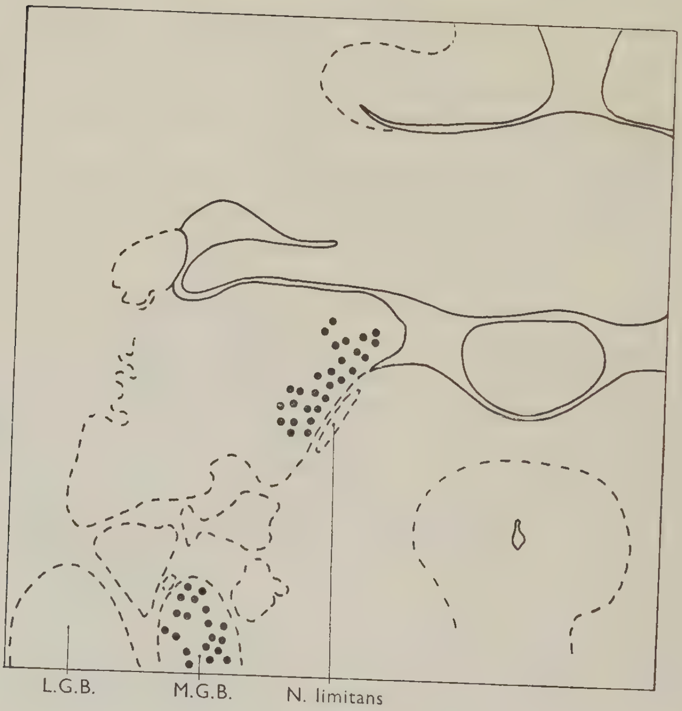

Fig. 3*b*.

Text-fig. 3. (*a*) The cortical lesion in Exp. 3, redrawn from Text-fig. 3 in the paper of Le Gros Clark & Russell (1939). (*b*) Reconstructed coronal section through the pulvinar in Exp. 3.

degenerated area, as it would appear in a strictly coronal plane, is shown in Text-fig. 3*b*. The area is rather larger than in the preceding cases, and the cell loss is much more complete; there are few residual neurons and an intense gliosis. In view of Chow's suggestion that the middle or posterior part of the superior temporal gyrus might be the projection area of the anterior part of P3, the whole nucleus was carefully examined. However, no certain evidence was found of degeneration in the anterior moiety of P3.

The observations made in these three experiments have been supplemented by re-examination of sections prepared in an earlier study of the temporal lobe (Le Gros Clark, 1936). In this investigation, the cortical lesions in two of the experiments, C and E, involved the anterior part of area 22 (TA), though less extensively than in our own experiments. In both cases, a careful study of the pulvinar region shows a slight diminution in cell density, together with some shrinkage of the surviving cells, in that part of P3 affected in our experiments. However, though they are confirmatory, these changes are minimal, and thus by themselves could hardly be expected to present positive evidence of a temporal projection of the pulvinar.

In Exps. A and B of the earlier study of the temporal lobe, the cortical ablations were in the middle and posterior portions of the superior temporal gyrus respectively. Examination of sections through the rostral half of the element P3 shows no certain evidence of retrograde cell atrophy. This negative observation is of significance in view of Chow's suggestion that this portion of the pulvinar may project to the superior temporal gyrus posterior to the projection area of the caudal half of P3.

HUMAN CASE REPORT

This case is presented by courtesy of Dr Ritchie Russell. The relevant history is as follows:

On 21 February 1945, Private G. received a penetrating head wound in the left fronto-temporal region. When first examined on the same day, he was semicomatose, with a flaccid paralysis of the right arm and a paresis of the right leg, and bilateral Babinski's sign. Next day, the wound was explored and excised. A bony defect 1 × 1 cm. was enlarged to expose a dural rent, through which pulped brain was removed to a depth of 3–4 cm. The ventricle was not encountered, though the surgeon considered that it had probably been traversed by fragments of missile seen in X-rays and not removed at operation.

Post-operatively, there was a profound aphasia, with total loss of propositional speech and apparent loss of comprehension. There was an incomplete left oculomotor palsy and a moderate right lower facial weakness. There were bilateral Babinski's signs, with diminished tone in the right arm. An electroencephalogram made 3 weeks after injury showed a generalized disturbance of medium voltage 3–6 cyc./sec. waves in all leads, tending to be episodic; and also a focal 3 cyc./sec. disturbance in the left mid-temporal region. Only the focal activity persisted in a later record.

Progress was initially good and all neurological disabilities improved, but in May 1945, following a lumbar puncture, the patient suddenly developed signs suggesting a ruptured para-ventricular abscess, with purulent but sterile cerebrospinal fluid. These signs entirely subsided under chemotherapy. However, though no neurological sequelae persisted, the subsequent course was unhappy and, in 1950, terminated in suicide.

Neuro-anatomical study: The brain was hardened in formalin and cut in coronal sections after being photographed. Injury was found to be confined to the left temporal pole and the corresponding lateral orbito-frontal region (Text-fig. 4*a*). Damaged areas include: (i) the superior temporal gyrus, which is destroyed from its anterior extremity to the level of the central sulcus; presumably the areas affected are 38 and 22 (TG and TA). (ii) The rest of the temporal pole, as is shown in Text-fig. 4*a*; the caudal extent of the lesion is much reduced in the middle and inferior temporal gyri. The uncus is intact. (iii) The postero-lateral part of the orbital surface of the frontal lobe: this lesion extends to the anterior border of the insula. (iv) The head of the left caudate nucleus in which an old, gliotic cavity is seen (Text-fig. 4*b*). This is doubtless the capsule of the paraventricular abscess diagnosed in 1945. The internal capsule appears somewhat shrunken; no other deep or superficial damage could be detected.

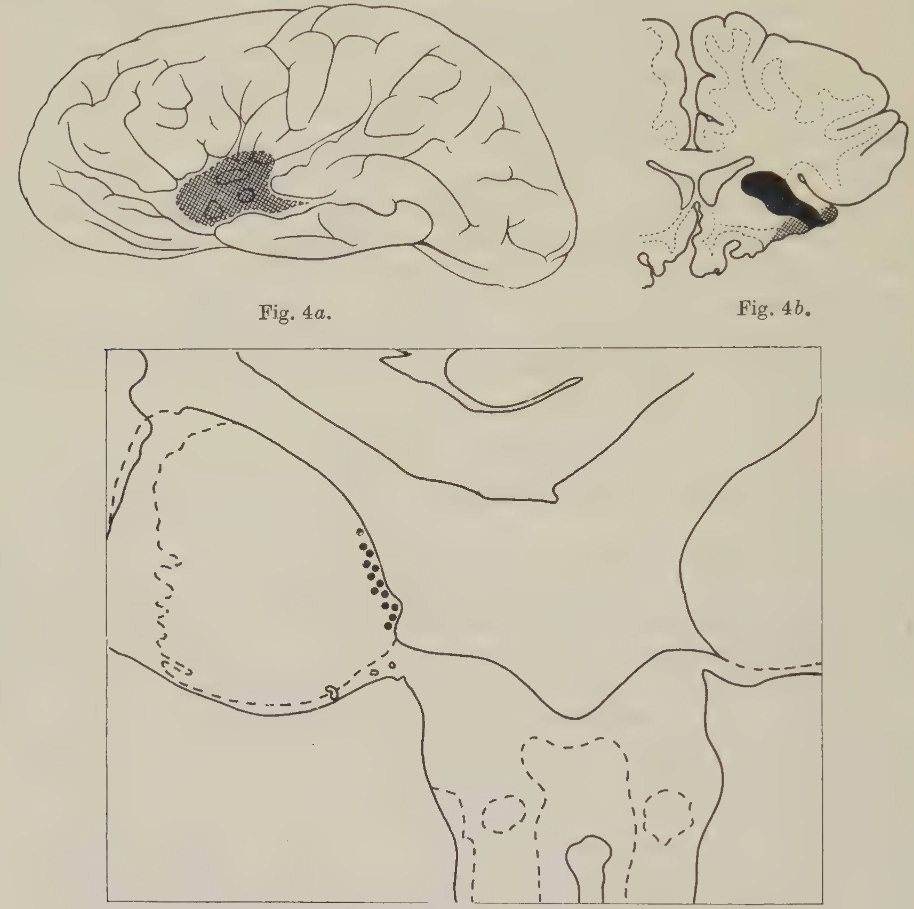

Fig. 4*a*. Fig. 4*b*.

Fig. 4*c*.

Text-fig. 4. (*a*) Lateral aspect of the human brain, drawn from a photograph. The brain is viewed from the lateral aspect and slightly from below. (*b*) Coronal section of the same brain at the site of the lesion, showing the abscess cavity; drawn from a photograph. In these figures, damaged cortex is indicated by cross hatching; the abscess cavity is shown in solid black. (*c*) Coronal section through the pulvinar region of the same brain, drawn with a projection apparatus.

Slices of the diencephalon were embedded in celloidin, cut at 30 μ, and stained with cresyl violet. The pulvinar on the left side shows a definite area of gliosis and cell loss. This appears (Text-fig. 4*c*; Pl. 1, fig. 2*a*) as a subpial zone, nowhere very deep, on the supero-medial aspect of the nuclear mass P3. At one point, the gliotic area is raised up to form a small eminence. Comparison with the normal side makes it probable that this represents part of a more extensive area of cell degeneration which has in the course of time become shrunken elsewhere to a flattened plaque of glial tissue.

DISCUSSION

The evidence presented in this report confirms and extends Chow's discovery of a projection of the pulvinar to the temporal pole in the macaque. As he has stated, the affected pulvinar area is a small portion of the medial nucleus P3; indeed, our findings suggest that the area is even smaller than his diagrams show, being restricted to the ventromedial part of the nucleus P3. The very limited extent of the cortical area receiving this thalamic projection probably explains the failure of previous workers to identify it. In this connexion it is interesting to note that a more extensive cortical ablation appears to increase the intensity of cell loss within the same area of the pulvinar, without a proportionate increase in the total area of the degeneration. This observation is in accord with the suggestion put forward by Rose & Woolsey (1948) that the essential projection area of each thalamic nucleus is surrounded by a fringe area which only receives collaterals from the nucleus, and that retrograde degeneration in a given thalamic nucleus is thus likely to be increased in intensity when ablation of its known cortical projection area is supplemented by excision of a belt of surrounding cortex. If this is so, it is clear that Chow's method of study (by the analysis of multiple large ablations) would lead to more intense and more obvious retrograde changes in the pulvinar than the small, selective lesions employed by previous workers and in our own experiments. Exp. 3, and Exp. A of the earlier temporal lobe series, provide no confirmation for Chow's suggestion that the anterior part of P3 is represented in the middle or posterior parts of the superior temporal gyrus.

The human case studied appears definitely to confirm the observations made on the monkey. The possibility has been considered that the gliosis and cell degeneration seen in the left pulvinar might be a sequel to the meningitis referred to in the case history. However, no similar lesions are found elsewhere in the available sections of diencephalon and other parts of the brain and it is reasonable to infer, therefore, that the cell degeneration is a true retrograde atrophy related to the injury to the temporal cortex. It may thus be stated that in the human brain (as well as in the macaque) the pulvinar gives a projection to the temporal pole. The retrograde changes seen in the human pulvinar are in the supero-medial part of P3, while in the macaque the ventro-medial part of this element is degenerated. However, this apparent discrepancy may be explained by a re-orientation of the pulvinar as a whole in man consequent on its great increase in size.

Until more is known of the functions of the pulvinar, and of its afferent connexions, it is not profitable to speculate on the possible significance of the projection of the nuclear element P3 to the cortex of the temporal pole. It may be noted, however, that the area 22 (TA) of the temporal pole is cyto-architecturally similar to the parietal cortex which is known to be intimately linked with the pulvinar, and it is immediately adjacent to the primary acoustic area.

SUMMARY

1. The existence of a localized projection from the pulvinar (element P3 of Aronson & Papez) to the cortex of the temporal pole in the monkey has been confirmed in five experiments with small discrete cortical lesions.

2. A human case demonstrating a similar relationship between the pulvinar and the temporal pole is presented.

I wish to thank Prof. W. E. Le Gros Clark for the initial planning of this work, and for much assistance at all stages of the experiments and their interpretation; Dr W. Ritchie Russell, for permission to report the human case; Mr E. A. Thompson and Mr L. D. Parke for excellent histological preparations; and Miss C. Court and Mr A. W. Dent for the drawings and microphotography.

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EXPLANATION OF PLATE

Fig. 1*a*. Microphotograph (×100) of the area of retrograde degeneration in the pulvinar in Exp. 1 showing cell atrophy and gliosis (cf. Text-fig. 1). Methylene blue stain.

Fig. 1*b*. Microphotograph showing the corresponding region of the pulvinar on the unoperated side.

Fig. 2*a*. Microphotograph (×64) of the area of retrograde degeneration in the pulvinar in the human brain, showing cell atrophy and gliosis (cf. Text-fig. 4). Cresyl violet stain.

Fig. 2*b*. Microphotograph showing the corresponding region of the pulvinar on the uninjured side.

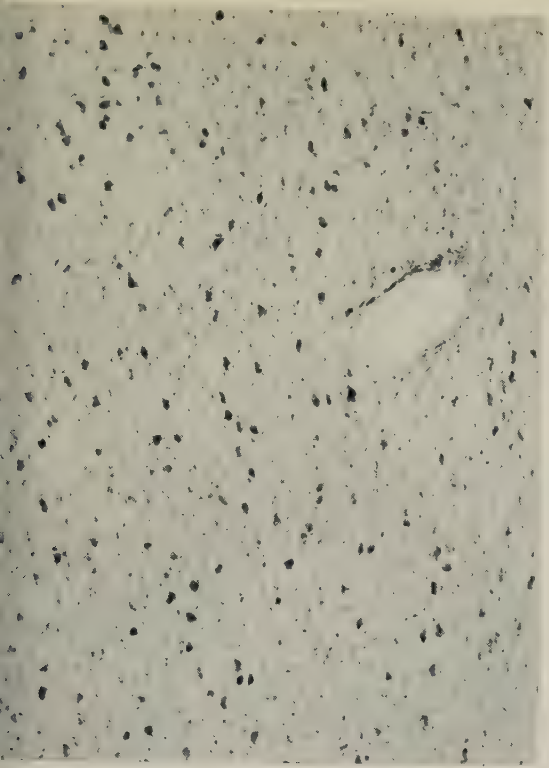

Fig. 1*a*

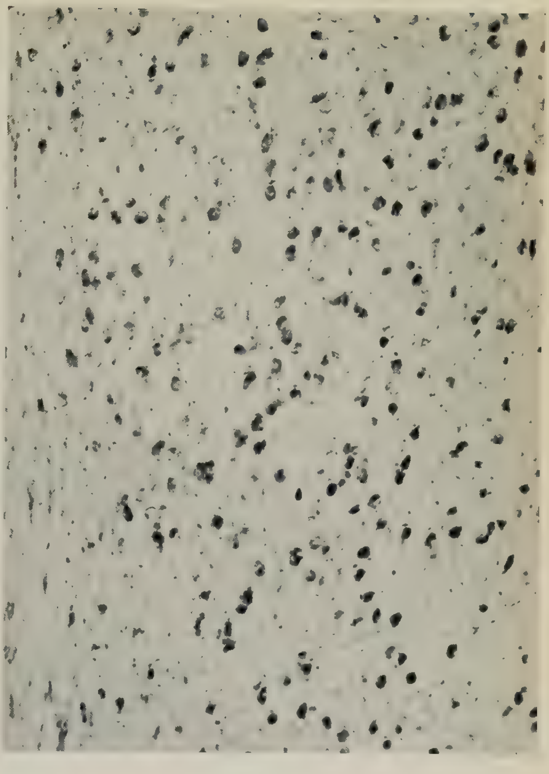

Fig. 1*b*

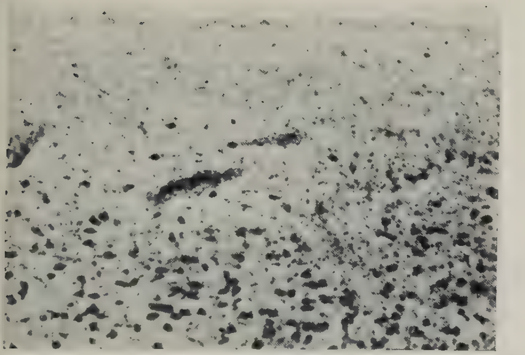

Fig. 2*a*

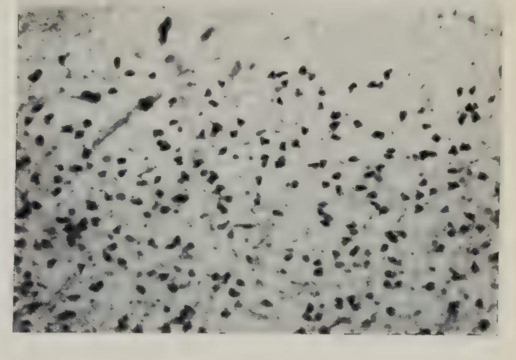

Fig. 2*b*

IMPSON—The projection of the pulvinar to the temporal lobe

CELL REPLACEMENT IN THE EPIDERMIS AND SEBACEOUS GLANDS OF THE MOUSE

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I. INTRODUCTION

In adult mice, which maintain a relatively constant weight, mitotic activity is evidently concerned merely with cell replacement. Since in normal circumstances the tissues and organs do not vary greatly in size, it must follow that the rates of mitosis and of cell wastage are delicately balanced. However, it has been demonstrated that in the various tissues of the mouse the mitotic rate is not constant, but varies widely from hour to hour in a diurnal rhythm (Bullough, 1948*a*) and, in the female, from day to day in an oestrous rhythm (Bullough, 1946). It has also been shown that in abnormal circumstances such as heavy muscular exercise, underfeeding, or other physiological stresses a deep mitotic depression may develop (Bullough, 1948*b*, 1949).

An attempt has therefore been made to determine the effects on tissue size of wide variations in the rate of cell replacement, and so to gain some insight into the manner in which the balance between cell loss and cell replacement is maintained. The skin was chosen for this study since its epidermal components normally have a relatively high rate of cell division, and since it has already been shown by Bullough & Eisa (1950) that it is possible to induce a pronounced depression in the epidermal mitotic rate by means of restricted diets.

II. MATERIALS AND METHODS

(1) *The mice*

The animals studied, all Strong's CBA males between 3 and 5 months old, were maintained on a graded series of restricted diets in order to induce a graded series of mitotic rates. At the beginning of the experiments they were all large animals in good health, having been reared since birth on a liberal diet of commercial rat cake with cod-liver oil, flaked maize, and dog biscuit. During the experiments they were fed only on rat cake, and previous observations had established that in the presence of plenty each mouse would eat, on the average, 3·6 g. of this cake daily. There were six groups, each containing five animals, which received respectively 3·8 g. of rat cake per mouse per day (105 % diet), 3·4 g. (95 % diet), 3·0 g. (85 % diet), 2·6 g. (75 % diet), 2·2 g. (65 % diet), and 1·8 g. (55 % diet). Abundant drinking water was always present. The mice were maintained on these diets for 4 weeks, and then, to arrest developing mitoses in the metaphase, each animal was injected with 0·1 mg. colchicine at 10·00 hr. and killed at 15·00 hr.

For details of the composition of the rat cakes, of the weekly changes in body weight, and of the final liver glycogen concentrations and blood sugar levels reference should be made to Bullough & Eisa (1950).

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* Sorby Fellow of the Royal Society.

(2) *The skin*

After the animals had been fixed whole in Bouin's alcoholic fluid, small pieces of skin were taken from the antero-dorsal region of the body over the scapulae, embedded in ester wax (Steedman, 1947), sectioned sagitally at 7 μ, and stained in Ehrlich's haematoxylin and eosin.

In the case of the epidermis the numbers of mitoses were counted in unit section lengths of 1 cm., only mitoses in the metaphase being included. From each skin sample ten such counts were made, and an average was taken. The epidermal thickness was then measured from the base of the stratum germinativum to the free surface of the stratum corneum, and again ten measurements were made from each skin sample and an average taken. The figures obtained in these ways from each experimental group were then analysed statistically.

In the case of the sebaceous glands similar measurements were made of the mitotic rate and of the amount of glandular material present. To determine the mitotic rate, the total number of cells arrested in the metaphase was counted in a total of ten successive sections of alveoli, and this procedure was repeated ten times for each skin sample and an average taken. In estimating the amount of glandular material, the method used was that devised by Ebling (1948), the total number of sebaceous cells (sebaceous cell count) and the total number of alveoli (alveolar count) being counted in a section length of 15 mm. from each skin sample. In addition, in three of the experimental groups, the total areas of the glands in a 15 mm. length of section were estimated by measurements made with a planimeter from outlines traced by means of a camera lucida. The figures for each experimental group were then treated statistically.

III. RESULTS

(1) *Epidermis*

After 4 weeks of the experimental conditions, it was found that mitotic activity in the antero-dorsal epidermis was depressed in all mice maintained on 85 %, or less, of the normal food intake (Table 1). However, as in the ear (Bullough & Eisa, 1950), the relation between mitosis and diet was not direct, and the figures obtained can be expressed as a sigmoid curve in which the greatest fall in the mitotic rate accompanies a reduction from about 80 to about 70 % of the normal diet. In the two lowest

Table 1. *Changes in the antero-dorsal epidermis of adult male mice after 4 weeks of restricted diets*

Diets expressed as percentages of normal food intake	Numbers of mitoses per unit length (1 cm.) of epidermis cut 7 μ thick		Thickness of epidermis in μ	
	Mean	Standard error	Mean	Standard error
55	4·5	0·34	16·8	0·2
65	4·6	0·23	16·0	0·7
75	7·6	0·52	16·9	0·6
85	16·6	1·16	16·4	0·6
95	19·5	1·46	16·4	0·2
105	20·7	1·24	16·3	0·3

fed groups maintained on 65 and 55 % diets respectively the epidermal mitotic rate was reduced to about 25 % of normal.

However, in marked contrast, it can also be seen from Table 1 that the thickness of the epidermis remained approximately constant in all groups. This indicates clearly that even when the epidermal mitotic rate is maintained for a month at a very low level, there is no reduction in the number of epidermal cells. The only obvious explanation is that the rate of cell loss must be reduced in exactly the same degree as the rate of cell replacement.

(2) *Sebaceous glands*

These glands are of the holocrine type, which means that the secretion is produced by destruction of their cells. Replacement takes place by mitotic activity of cells adjacent to the basement membranes of the glands, and the results of mitotic counts in these regions are shown in Table 2. Again it can be seen that the degree of mitotic activity in the two lowest fed groups was approximately a quarter of the normal, and that the greatest fall in mitotic activity accompanied the reduction from about 80 to about 70 % of the normal diet.

Table 2. *Changes in the sebaceous glands in the antero-dorsal skin of adult male mice after 4 weeks of restricted diets*

Diets expressed as percentages of normal food intake	Numbers of mitoses in ten gland sections		Sebaceous cell count in 15 mm. of section		Alveolar count in 15 mm. of section		Total alveolar area (sq.μ) in 15 mm. of section	
	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error
55	0·18	0·05	350·4	31·8	54·6	3·4	76,506	9503
65	0·24	0·05	360·6	23·7	46·0	2·4	73,873	4467
75	0·52	0·08	385·6	6·4	58·0	2·1	—	—
85	0·94	0·10	365·4	23·3	55·0	2·9	—	—
95	0·88	0·08	387·0	31·8	54·6	4·2	—	—
105	0·86	0·06	422·4	33·1	54·8	2·0	87,887	7331

Table 3. *Mitotic rate, cell size, and alveolar size in the sebaceous glands of adult male mice after 4 weeks of restricted diets*

(The figures are derived from measurements given in Table 2.)

Diets expressed as percentages of normal food intake	Numbers of mitoses per 0·1 sq.mm. of gland section	Average cell-size (sq.μ)	Average number of cells per alveolar section	Average alveolar area (sq.μ)
55	1·29	215	6·4	1388
65	1·49	204	7·8	1612
75	3·37*	—	6·6	—
85	6·27*	—	6·6	—
95	5·81*	—	7·1	—
105	5·38	208	7·7	1595

* Calculated on the assumption that the average alveolar area in these groups was 1500 sq.μ.

With regard to the quantity of glandular material present, no significant variations were found in either the sebaceous cell counts, the alveolar counts, or the total areas of the alveolar sections (Table 2). For an understanding of these measurements reference may be made to the quantities derived from them which are shown in Table 3. From this it can be seen that the size of the sebaceous cells (mean total area of alveoli divided by mean sebaceous cell count) shows no significant variation from group to group, and that the alveolar size as estimated either by the average number of cells per alveolar section or by the average area (mean total area divided by mean alveolar count) also remains constant.

It may therefore be concluded that no significant variations occurred in sebaceous cell size, alveolar size, or alveolar number as a result of the reduced mitotic rate.

IV. DISCUSSION

The most important conclusion arising from these results is that the rate of cell loss by keratinization, like the rate of sebum secretion from the sebaceous gland, is determined primarily by the rate of mitotic activity in the deeper cell layers. However, further consideration of this question indicates clearly that this conclusion only holds within certain limits, wide though they may be. In conditions of chronic starvation the mitotic activity of the epidermis may be almost eliminated (Bullough, 1949), and it is well known that, as a result, tissues may shrink considerably in size before death supervenes. Similarly, at the other end of the scale, it is known that increases in tissue size may follow powerful stimulation of the mitotic rate. This occurs in mouse epidermis when the mitotic rate is increased from five- to tenfold and the thickness threefold by the irritating action of croton oil (Bullough, unpublished), and in rabbit epidermis when papillae develop in response to local paintings with androgenic hormones (Montagna, Kenyon & Hamilton, 1949). A considerable increase in the amount of sebaceous material present in the skin also follows the injection of large quantities of androgenic hormones into rats (De Graaf, 1946; Ebling, 1948).

It is now well known that the androgenic and oestrogenic hormones exert a powerful influence on the rate of cell replacement, which is especially obvious in the accessory sexual organs and the secondary sexual structures. The oestrogenic hormones also have a powerful action on the epidermis and the sebaceous glands, and considerable changes in both mitotic rate and tissue size occur naturally during the oestrous cycle of the mouse and rat. If the mitotic rate of the antero-dorsal epidermis of the well-fed adult male is represented by the figure 20, that of the well-fed female varies between 15 on the first day of dioestrus and 75 in pro-oestrus (Bullough, 1946, and unpublished). This variation is so great that changes in epidermal thickness follow, the highest values being found during early oestrus (H. F. Bullough, 1943). Changes in epidermal thickness can also be induced by injections of oestrone (H. F. Bullough, 1947). In the sebaceous glands variations occur both in the mitotic activity (Bullough, 1946) and in the quantity of sebaceous material (Ebling, in the press) during the oestrous cycle. Increases in the mitotic rate and in the size of the sebaceous glands can be induced experimentally by treatment with oestrogens (Bullough, 1946; Ebling, 1951).

However, while both androgens and oestrogens increase the rate of cell replacement in the skin to such a degree that increases in the numbers of epidermal and sebaceous cells follow, the actions of these two groups of hormones differ in detail. During continued administration the effects of the androgens are evidently moderate but prolonged, while those of the oestrogens are great but transitory. The oestrogenic stimulation of the mitotic rate persists for only a day or two at the most (Bullough, 1946; H. F. Bullough, 1947), and with continued treatment the epidermis thins, the hair drops out, and the sebaceous glands shrink (Hooker & Pfeiffer, 1943; Ebling, 1948, 1951). The reasons for this are not yet understood, but similar changes are known to occur after prolonged administration of adrenocorticotropic hormone (Baker, Ingle, Li & Evans, 1948) or of cortisone (Castor & Baker, 1950). It is obvious from the present results that the mitotic depression induced in these cases must have been very severe.

The evidence at present available suggests that two sets of factors should be considered in relation to the control of cell replacement. First, each tissue seems to have an intrinsic capability for cell division which is related to its normal rate of cell loss. Thus the epidermis and the sebaceous glands show high mitotic activity and considerable cell loss, while tissues such as striped muscle and brain show neither. Secondly, it is now evident that, within limits, the rate of cell loss in a tissue can be adjusted to that of cell production. It follows incidentally that in well-fed animals the mitotic rate is considerably higher than is necessary for the normal maintenance of the tissues, and this is consistent with the observation (Tannenbaum, 1947) that even with a restricted diet mice continue to live in good health.

V. SUMMARY

1. The mitotic rates in the epidermis and in the sebaceous glands of adult male mice were depressed in various degrees by means of a graded series of restricted diets. In both cases it was found that the relation between the mitotic rate and the food intake could be expressed by a sigmoid curve, and that the greatest mitotic depression occurred with a reduction from about 80 to about 70 % of the normal diet.

2. With diets between 55 and 65 % of normal the mitotic rates were reduced to about a quarter, but neither the thickness of the epidermis nor the size of the sebaceous glands was affected.

3. Although it is recognized that each tissue appears to have an intrinsic capability for mitosis which is related to its normal rate of cell loss, it is suggested that within relatively wide limits the rate of cell loss is itself closely dependent on the rate of cell production.

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THE PATTERN OF MOTOR INNERVATION IN MAMMALIAN STRIATED MUSCLE

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INTRODUCTION

It has long been known that the motor nerve fibres entering a muscle may branch profusely to supply many muscle fibres (Ramón y Cajal, 1881, 1909), but little information is yet available on the mode of distribution or the extent of overlap and intermingling effected within a muscle by these nerve fibres and their terminals. Further knowledge of these features of the pattern of innervation in mammalian striated muscles seems desirable in order to provide a more precise anatomical background for studies of muscle function.

The present report is based upon the examination of the neural pattern in selected muscles of the rabbit and in the extrinsic ocular muscles of two macaque monkeys.

MATERIALS AND METHODS

The anatomical material was obtained from eighteen young adult rabbits and from two macaque monkeys.

The muscles examined include the lumbrical and other intrinsic plantar muscles, the extensor digitorum longus, tibialis anterior, soleus, diaphragm, sterno-mastoid, crico-thyroid and the extrinsic ocular muscles.

The main histological method used was the technique of slow intravenous perfusion of methylene blue into the anaesthetized animal. The details of this technique and its application to neuro-anatomical studies have already been described (Feindel, Sinclair & Weddell, 1947; Feindel, 1948). This method, if conditions are optimal, gives selective staining of intramuscular axis cylinders and nerve endings. It is thus possible, after fixation and clearing, to map out the general neural pattern and to examine details of innervation in whole preparations of small muscles such as the lumbricals and extrinsic ocular muscles. Because the dye is perfused throughout the animal a range of preparations illustrating the innervation of muscles from different parts of the body can be obtained and compared. The larger muscles, however, must be divided into smaller segments before the details of their innervation can be determined. The segments which can be usefully examined are, nevertheless, relatively large compared with the extent of tissue available for study in the usual paraffin or frozen sections.

The neural elements in the crico-thyroid and sterno-mastoid muscles were stained by injecting a dilute solution of methylene blue directly into the muscle (Weddell, Harpman, Lambley & Young, 1940). Although this method inevitably gives considerable mechanical distortion of the neural pattern in such a tissue, material so obtained was nevertheless of considerable value for examining the arrangement of pre-terminal motor fibres and of end-plates.

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In muscles too large to be examined histologically in the form of whole preparations, the neural patterns were examined by dissection under a low power binocular microscope. The muscle fibres were carefully teased apart to display the main nerve trunk and many of its smaller branches. The exposed nerves and the muscle fibres around them were then moistened at intervals with a 0·25 % solution of osmium tetroxide, which blackens the nerves and renders visible the finer nerve bundles which proceed from them. The muscles were then allowed to clear in pure glycerol. After clearing it is possible, particularly in strap-like muscles, to display extremely fine nerve bundles by carefully teasing the muscle fibres off their surface.

The crico-thyroid muscles from two rabbits were cut in serial sections and impregnated by the protargol silver method of Bodian after the following experimental procedures had been carried out. The muscles were prepared in one animal 6 weeks after section and removal of a segment from the left superior laryngeal nerve; in the other animal, 6 weeks following unilateral section and removal of a segment of the motor branch to the muscle from the pharyngeal plexus. Immediately before the animals were killed, the crico-thyroid muscles were examined electro-myographically.

OBSERVATIONS

Intrinsic plantar muscles

In intact lumbrical muscles prepared by the intravenous staining technique the course of nerve fibres can be examined from a point 25 mm. proximal to the muscle, up to their final terminations within the muscle. The remarkable complexity of the neural pattern as illustrated by an area of lumbrical muscle shown in Pl. 1, fig. 1, precludes the tracing of individual axons over any great distance. However, since such preparations provide for study considerably larger areas of the intramuscular neural plexus than have been previously reported in the literature, it seems worth while to describe an example in detail.

The general scheme of the neural pattern in the area of lumbrical muscle (Pl. 1, fig. 1) is brought out more clearly in the diagram (Pl. 1, fig. 2) based on the photomicrograph.

The course of the main nerve trunk is represented by the heavy black line, *A*–*X*–*B*, running through the centre of the diagram. A number of large nerve bundles branch off from this main nerve stem to form an intricate plexiform pattern. Near the mid-point (*X*) of this main nerve stem one large nerve bundle branches off to the left, and divides in turn to form smaller bundles, *D* and *E*, running to right and left. Another bundle of the plexus, marked *C*, is represented near the top left of the diagram. Still another bundle, labelled *F*, comes off the deep-lying portion of the main stem, between *X* and *B*. It may be noted that these secondary bundles continue beyond the field shown in the photomicrograph.

In this zone of muscle, twelve bundles of terminal fibres (indicated in the diagram, Pl. 1, fig. 2, by arrowed lines), which are derived from the main nerve stem and its secondary branches, give rise to sprays of motor end-plates.

All the nerve fibres do not travel to their terminations in the direction *A* to *B*, a fact suggested by the angle at which the individual fibres or small bundles leave the main stem or its branches. Examination under high magnification shows that some of the branches are made up chiefly of nerve fibres which have an upward direction, i.e. from ***B*** to ***A***.

The plexiform nature of the pattern is illustrated at the forks of the main nerve trunk and its secondary branches. At such forks, axon bifurcation frequently takes place and each collateral so formed can often be traced into different branches of the fork. This clearly makes it possible for one nerve fibre to supply endings which may lie in different regions of the muscle. Axon branching also takes place in the course of a nerve bundle not in the region of a fork. This is illustrated by Pl. 3, fig. 11, where the arrow indicates branches which continue for a distance in the same nerve bundle. It can also be seen that some of the fibres in the branches of the main nerve stem (Pl. 1, fig. 1) are derived from sources other than the main stem. For example, the branches *D* and *E* contain in common some nerve fibres not derived from *A–X–B*.

The terminal fibres and motor end-plates are disposed for the most part in groups which may consist of as many as fifteen or twenty endings. Less commonly, groups of two or three endings are seen: these are usually close to the larger groups of terminals. Isolated endings are rare, and when present, are borne upon fibres which have their origin near the terminal distribution of a small nerve bundle, and only unusually from a large plexus bundle. The sprays of motor end-plates are usually grouped in circumscribed zones at some distance from one another, the intervening zones being free from end-plates and often from nerve fibres as well.

The pattern shown by these terminal sprays of motor endings is such that several different axons of a nerve bundle supply end-plates in the same zone of muscle. Many of these axons, when traced back from the end-plates, are seen to remain separate from each other, although each axon may commonly give off collaterals which run in other nerve bundles to other zones of the muscle. Some of the axons, however, branch in their preterminal course (Pl. 3, fig. 11), so that the number of end-plates in any given zone of muscle is greater than the number of axons in the nerve bundle supplying that zone.

The motor end-plates derived from any one axon supplying terminals in that zone may be arranged in a variety of ways. Most frequently, however, the individual endings of one axon terminate on scattered muscle fibres separated by intervening muscle fibres which are supplied by other axons of the terminal nerve bundle to that zone.

The resulting arrangement is such that each axon of the terminal nerve bundle appears to fan out and branch to supply throughout the zone end-plates which interweave with the endings of other axons of the same bundle. Conversely, if several adjacent motor end-plates are picked at random in a zone of muscle and their axons of supply traced proximally, it is usual to find that they are derived from different axons of the nerve bundle.

A similar arrangement of terminal sprays of motor end-plates is found in the crico-thyroid muscle (Pl. 2, fig. 6). Here again the muscle fibres are innervated by nerve bundles the axons of which fan out, divide and end on muscle fibres so that endings derived from one axon interweave with those derived from other axons of the same nerve bundle.

Additional examples of grouped end-plates from another lumbrical muscle are shown in Pl. 2, fig. 5. Nerve fibres from three different nerve bundles, labelled *A*, *B* and *C*, supply motor endings to muscle fibres in this region. Each nerve bundle approaches the area from a somewhat different direction as indicated by the arrows. The endings interweave in relation to the distribution of the muscle fibres which they supply. At the optical plane represented, for example, two terminals from bundle *A* supply muscle fibres on either side of a third muscle fibre which receives a terminal from bundle *B*. The terminal fibres to this zone of muscle are also arranged in sprays, the nerve bundles *A*, *B* and *C* giving off respectively four, two, and five end-plates. Careful examination showed no evidence of a muscle fibre in this zone having more than one axon supplying it. As in the preceding examples, it is to be noted that there is no arrangement whereby a solitary axon approaches a local area to supply end-plates to a group of contiguous muscle fibres.

Although this report is devoted primarily to a consideration of the pattern of motor nerve fibres in muscle, it need hardly be mentioned that many of the intramuscular nerve fibres are afferent in function. In nerve bundles of the intramuscular plexus these may be indistinguishable from nerve fibres derived from ventral roots. Nevertheless, two types of sensory fibres may be identified with reasonable certainty.

Scrutiny of the large nerve bundles shown in Pl. 1, fig. 1, will disclose occasional nerve fibres of larger diameter, which are 'giant' afferent fibres supplying muscle-spindles and tendon-organs.

A second type of fibre is indicated by arrows in Pl. 1, fig. 1. It is distinguished by its small size and its beaded appearance and by the fact that, unlike the larger intramuscular nerve fibres, it does not supply organized endings. Such fine beaded fibres occur infrequently in the muscle tissue proper, this being the only one found in the area of muscle pictured in Pl. 1, fig. 1. They may be seen more often in the connective tissue septa or sheath of the muscle.

Neural pattern in larger muscles

The general neural pattern in different muscles as demonstrated by methylene blue staining shows considerable variation. Neither the size and number of the nerves entering a muscle nor their sites of entry give much indication of their intramuscular distribution and pattern. In one muscle a small nerve entering as a single trunk at one point may arborize to cover a wide intramuscular region. In other muscles, as is well known, the zone of innervation may be restricted to part of the muscle so that other regions are almost lacking in nerve fibres and terminals.

A portion of the gross neural pattern within the sterno-mastoid muscle as displayed by dissection under a binocular microscope is shown in Pl. 1, fig. 4. The main nerve runs lengthwise through the muscle and at intervals gives off lateral branches. Some of these branches leave the main nerve trunk almost at right angles and subsequently divide into smaller nerve bundles. A proportion of these smaller nerve bundles take a recurrent course and 'anastomose' with bundles derived from neighbouring branches of the main nerve trunk (Pl. 1, fig. 4, arrows). It should be noted that such 'anastomoses' occur in relation to nerve trunks and nerve bundles, and not in relation to single nerve fibres, which retain their individuality within the intramuscular plexus. It is clear that not all the nerve fibres in one of the lateral branches are destined to end in the zone of muscle to which they are at first directed, since some may be recircuited by way of these anastomotic bundles to other parts of the muscle.

The gross neural pattern in the other large muscles which have been examined in this study (extensor digitorum longus (Pl. 1, fig. 3), tibialis anterior, soleus, and the diaphragm) varies to a greater or lesser degree from that just described, according to the arrangement of the muscle fibres within the muscle as a whole. 'Loops' formed by recurrent nerve fibres are seen in all these muscles although the number and size of such 'loops' varies considerably.

In segments of larger muscles stained by methylene blue, it is possible to trace the course of some individual axons for a considerable distance. Single axons have been seen giving rise to numerous branches which pursue a complicated, at first usually divergent, but later sometimes recurrent course through the intramuscular plexus. Such single axons thus give rise to end-plates in widely separated positions throughout the muscle segment. It is also possible to determine that the motor end-plates supplying contiguous muscle fibres in any given zone of innervation may be supplied by axons which approach by different routes through the intramuscular plexus as described with reference to the lumbrical muscles (Pl. 2, fig. 5). In all segments examined it is rare to find isolated motor fibres and end-plates.

The neural pattern in these larger muscles of the rabbit is therefore similar in essential features to that in the lumbrical muscles.

Crico-thyroid muscle

The crico-thyroid muscle, which is supplied by two separate nerves, the superior laryngeal and a branch from the pharyngeal plexus, has been included in this report because it has been stated that single muscle fibres in this muscle receive a dual motor supply (Exner, 1885).

Both main nerves to the muscle take part in the formation of a common intramuscular plexus, and histological examination of the crico-thyroid muscle suggests that its neural pattern is similar to that of the lumbrical muscles. Unfortunately, since the specimens of crico-thyroid muscle were stained by the local injection technique, it was not possible to define the course of the axons in relation to the whole neural pattern as in the case of the lumbrical muscles. However, well-stained sprays of motor end-plates in local zones of crico-thyroid muscle (Pl. 2, fig. 6) are similar to those found in lumbrical muscles. In the light of the analysis of these terminal groups in whole preparations of lumbrical muscles it seems justifiable to assume that, in the crico-thyroid muscle as well, these terminal sprays represent intermingling of end-plates which, in some instances at least, are derived from different parent axons of the intramuscular plexus.

Six weeks after unilateral section of the branch of supply from the pharyngeal plexus, normal motor end-plates were seen scattered throughout the partially denervated muscle. In a second experimental animal, 6 weeks following section of the superior laryngeal nerve, end-plates were again found scattered throughout the muscle. Each nerve thus gives rise to terminals which are distributed throughout the muscle, rather than to any particular zone of it.

Mutiple end-plates on single muscle fibres

From time to time, evidence of a morphological or physiological nature has suggested the innervation of individual muscle fibres by more than one axon. In the present study no direct histological evidence for this was found. Certain observations have been made, however, which have a bearing on this problem.

Where a group of muscle fibres innervated by a spray of fifteen to twenty intermingled end-plates is examined under the low power of the microscope, it often appears that one muscle fibre is supplied by two or even more individual axons and end-plates (Pl. 2, fig. 5). However, critical examination showed that, in the majority of cases, each end-plate was associated with a separate muscle fibre. This could be determined by examination of thick, cleared preparations under high magnification, when each individual end-plate, whether seen in surface (Pl. 2, figs. 9 and 10) or side view (Pl. 2, figs. 7 and 8), could be clearly defined in relation to its corresponding muscle fibre.

Nevertheless, in a few instances, single muscle fibres associated with two or more end-plates have been observed in preparations of crico-thyroid muscles (Pl. 3, fig. 11) and limb muscles (Pl. 3, figs. 12 and 13).

It is perhaps worth while to emphasize again that the occurrence of two or more end-plates on a single muscle fibre does not necessarily provide histological evidence for true multiple innervation by different parent axons. In examples observed in this study, such end-plates were always supplied from the same parent axon. In Pl. 3, figs. 12 and 13, the double end-plates are situated close together on the muscle fibre. The axon either divides near its termination (Pl. 3, fig. 13) or supplies two end-plates 'in series'. In an unusual example taken from the crico-thyroid muscle (Pl. 3, fig. 11), the terminal fibres supplying a double end-plate could be readily followed back to their point of division.

In whole specimens or larger areas of muscle prepared by the methylene blue techniques we have employed, it is usually possible to trace out the exact arrangement of such double terminals: in material prepared in the form of thin serial sections, it is rarely possible to do this. A picture illustrating this is taken from a paraffin section of muscle stained by the protargol method of Bodian (Pl. 3, fig. 14). The lower terminal is a double end-plate obviously supplied by one axon. The origin of the upper end-plate which lies on the same muscle fibre cannot be determined. In the light of the previous examples, it is not possible to assume that the multiple end-plates on this single muscle fibre are necessarily derived from different parent axons.

Nevertheless, the possibility that single fibres in the muscle have a dual innervation cannot be wholly excluded on histological evidence. In an attempt to throw further light on the matter, in two animals one or other of the motor nerves to the crico-thyroid muscle was sectioned unilaterally. Six weeks after these operations electromyographic studies of the partially denervated muscles were made immediately after sectioning the intact nerve. Spontaneous fibrillation action potentials were recorded from all parts of the affected muscles by using either very fine monopolar or concentric needle electrodes. At no point on or within the muscle was there electrical 'silence'. Immediately after section of both motor nerves no action potentials could be obtained from any position on or within the muscles on the unoperated side.

Neural pattern of extrinsic ocular muscles

The extrinsic ocular muscles differ distinctly in their neural pattern from the muscles considered above. The pre-terminal nerve fibres usually run in a direction approximately parallel to the muscle fibres. There appears to be less terminal intermingling of the nerve bundles and more variation in size of individual terminal fibres (Pl. 4, fig. 18) than in other muscles examined in this study. The density of nerve fibres and endings in some zones of the muscle is particularly striking.

The nerves supplying the ocular muscles are also distinguished by their mode of terminal branching. The terminal branches in the limb muscles are most commonly arranged as small bundles of intermingling nerve fibres each of which supplies a spray of end-plates in a zone of muscle (Pl. 2, figs. 5 and 6). Isolated terminal fibres and end-plates are unusual and an axon supplying more than one end-plate to the same muscle fibre is a rarity.

In contrast, both these features are frequently observed in the ocular muscles. Single isolated nerve fibres are as common as small nerve bundles (Pl. 4, figs. 15, 16 and 20). Characteristically, a nerve fibre supplies a number of pre-terminal fibrils ending in plaque-like terminals, many of which are applied to the same muscle fibre (Pl. 4, figs. 15 and 16). Other nerve fibres can be traced along a muscle fibre to their termination in complex endings composed of a series of irregular knobs (Pl. 4, fig. 15, fibres 2 and 3; and fig. 17). Endings of much the same appearance as ordinary motor end-plates found in limb muscles are also present in the ocular muscles (Pl. 4, fig. 21). Near the insertion of the muscle a third, more complex, type of ending is found, in the form of terminal fibrils which split repeatedly to supply plaques and knob-like endings (Pl. 4, figs. 18 and 19). As with the nerve endings noted in Pl. 4, figs. 15 and 16, these also extend for a considerable distance along the muscle fibre.

DISCUSSION

General features of the neural pattern

With the exception of the extrinsic ocular muscles, all the muscles examined in this study appear to have neural patterns which are essentially similar in their main features. The intricate plexiform pattern formed by the intramuscular nerve trunks and bundles provides anatomical pathways for much mixing and interweaving of the nerve fibres during their distribution within the muscle. At the points of subdivision of the nerve trunks making up the intramuscular plexus, groups of individual fibres become segregated into nerve bundles which course in divergent directions. Very commonly fibres undergo division immediately proximal to such a fork and the collaterals take divergent pathways.

An increase of the fibre-counts immediately after division of a nerve trunk, so that the sum of the fibres in the two smaller bundles is greater than the number of fibres in the parent nerve trunk, does not necessarily imply that the collaterals formed by dichotomy take divergent paths along two different nerve trunks. As noted by Eccles & Sherrington (1930), increase of fibres by division occurs along parts of a nerve trunk not undergoing separation into smaller bundles. Occasionally geminal fibres can be seen coursing together for short distances or even running into the same nerve bundle when an alternative diverging bundle is available. However, the most common event when the fibres of a nerve trunk are sorted out into two different bundles appears to be a divergence of collaterals, and certain knowledge of this can be obtained by directly tracing the divergent path of the fibres, as in the present studies, or by showing that action potentials can be conducted from one collateral branch to the other by way of their common junctional stem (Adrian, 1925; Cooper, 1929).

These anatomical features, the dichotomy of fibres and the divergence of their collateral branches, make it possible for one parent fibre in the main nerve to supply many terminals, some of which may be applied to individual muscle fibres scattered throughout different parts of the muscle. The anastomoses of intramuscular nerve trunks and branches, depending upon the extent to which these are present in any particular muscle, enable an even more complex distribution of nerve fibres to take place.

The sprays of pre-terminal fibres and end-plates given off from the intramuscular nerve bundles usually innervate a group of contiguous muscle fibres. The individual endings of such sprays are, however, frequently derived from different axons.

It may be noted in passing that a different pattern of innervation is presented by the large sensory receptors in muscle, of which the neuromuscular spindles and the tendon end-organs are the main types. In contrast to the complexity of branching and intermingling of the motor fibres, the large afferent nerve fibres which supply these end-organs are distinguished by absence or paucity of branching (Feindel, 1949). These sensory nerve fibres therefore provide a direct restricted pathway between a local zone of muscle and the spinal cord. The fine, beaded, non-myelinated fibres which supply free nerve endings to the connective tissue septa or sheath of a muscle and to muscle spindles are probably related to pain sensibility in muscle (Feindel, Weddell & Sinclair, 1948).

Overlap and intermingling of nerve endings

The terminals derived from different axons intermingle and overlap to a marked degree, somewhat more in the intrinsic plantar than in the lumbrical muscles. Such overlapping is probably at its greatest in the distal muscles of the limb. These findings provide an anatomical basis for the physiological observations of Sherrington (1892).

Sherrington found that in the macaque monkey the lumbricals and interossei of the foot receive a segmental supply from the 7th, 8th and 9th subthoracic roots of a post-fixed plexus and from the 7th and 8th of a pre-fixed plexus. Stimulation of the anterior roots of any of these segments resulted in contraction of the lumbricals and interossei. He also observed that in the monkey, cat and rabbit the neural segments supplying the distal part of the limb 'are so intimately fused in the plantar region that it is difficult to pick out from the rest any one muscle which shows more of this or that constituent'. Sherrington further demonstrated that this intermingling could be defined in terms of even smaller units, since stimulation of different filaments of the roots reproduced visible contraction in the same muscles as did stimulation of the entire root. Indeed, in the monkey, stimulation of the highest of nine rootlets of the 8th subthoracic segment produced the same type of movement of the hallux as stimulation of the lowest of four rootlets of the 9th segment (Sherrington, 1892).

From this evidence it would seem necessary for intermingling of the motor fibres in such distal muscles to occur to such an extent that all parts of the muscles would be supplied by terminals from different rootlets. From the present histological study it is apparent that the neural pattern of these muscles features a degree of overlap and intermingling of intramuscular nerve fibres which is in keeping with the diffuse physiological representation of each motor rootlet throughout these muscles.

So far as they go, the histological observations made in this study on larger muscles also would support the idea of interweaving of axonal terminals and the separation and scattering of muscle fibres belonging to the same motor unit.

The gross neural pattern certainly varies in the different muscles we have examined, but it remains approximately the same in a given muscle from different rabbits. Although the number of muscles we have studied is small, in all of them the gross neural pattern seems to adapt itself to the arrangement of the muscle fibres with the muscle as a whole, and at the same time allows, by the provision of 'loops', for the wide distribution and interweaving of motor units.

That electrical stimulation of single axons may give contraction of muscle fibres in separated parts of a muscle has been shown in the cat by Adrian (1925) for the tenuissimus muscle and by Cooper (1929) for the sartorius muscle. Van Harreveld (1946) avulsed one of the spinal nerves containing fibres supplying the sartorius muscle in the cat and removed the muscle 2 weeks later. Transverse sections showed intermingling of normal and atrophic muscle fibres. He concluded that muscle fibres of motor units in the sartorius muscle do not form a single close bundle, but that elements of the unit are scattered, and intermingled with the muscle fibres of other units.

The innervation of single muscle fibres by more than one axon, whether from the same or from a different spinal segment, does not appear to have been substantiated physiologically or histologically in mammalian muscle (Fulton, 1926; Hinsey, 1934; Denny-Brown & Pennybacker, 1938). In the present study, in spite of the intimate interweaving of terminals supplied by different axons, no histological evidence has been obtained that individual mammalian muscle fibres receive end-plates derived from more than one axon. On the contrary, multiple end-plates associated as a rarity with the same muscle fibre were always found to be derived from the same parent axon. In sectioned material the ease with which multiple end-plates may be mistaken for 'multiple' or plurisegmental innervation has already been pointed out by Kulchitsky (1924) and Denny-Brown & Pennybacker (1938).

Neural pattern and electromyography

Although anatomical information relating to the mode of innervation of human skeletal muscle is meagre, there is some evidence to suggest that in many human muscles the arrangements may resemble those just shown to exist in certain muscles in the rabbit. It was shown by Duchenne (1867) and Frohse (1898) that there are in many human muscles complicated intramuscular plexuses of nerve bundles which resemble those described in the rabbit. It has also been shown by Wohlfart (1935, 1949) that in denervation of human muscle due to disease of the motor neurons the unit of muscular atrophy does not correspond to the muscle fasciculus.

The mode of distribution of motor units in large human muscles cannot be determined by the anatomical methods used in this investigation. The technique of electromyography may possibly prove of particular value in this connexion. The present study suggests, however, that anatomical features of the neural pattern of striate muscles may render the interpretation of electromyographic records more difficult than has generally been supposed.

This is illustrated by a consideration of the relation of electromyographic electrodes to the neural pattern in a lumbrical muscle. In Pl. 1, fig. 1, the small circle *M* has a diameter equivalent to 80μ which is somewhat greater than the tip of the monopolar electrode used by Jasper (1946). The larger circle *B* has a diameter equivalent to 500μ which would correspond to the outside diameter of a No. 23 hypodermic needle, which is commonly employed in making co-axial electrodes. Regardless of the exact positions of such electrodes it is clear that in the lumbrical muscle they would rest among a number of motor units and would frequently contact large and small nerve bundles conveying impulses to or from distant regions of the muscle. Both types of needles would clearly sample the electrical activity derived from contracting muscle fibres for a much greater distance around their tips. In the case of the co-axial electrode this distance is as much as 1 cm. (Weddell, Feinstein & Pattle, 1944). The total width of the area of muscle illustrated in Pl. 1, fig. 1 measures only about 0·1 cm., so that on the basis of a recording zone 1 cm. in diameter, the co-axial electrode would be capable of sampling a volume of muscle tissue of which the diameter would be ten times that of the total muscle area represented in Pl. 1, fig. 1.

It may be argued that the density of the neural elements at any given point in many human muscles will be less than in the lumbrical muscle of the rabbit. However, these anatomical observations would indicate that the features of the neural pattern are of significance in determining the nature and particularly the variability of the electrical record obtained from different muscles and from adjacent zones of the same muscle. They should be borne in mind when attempting to analyse complex electromyographic recordings.

For example, it is known (Weddell *et al.* 1944; Feinstein, 1946; Petersén & Kugelberg, 1949) that discrete single action potentials are obtained only with difficulty from the facial and laryngeal muscles. Records from these muscles are characterised by complex polyphasic discharges. This suggests that the axonal terminals of the different motor units within these muscles can overlap to such a degree as to prevent the activity of any one unit from being isolated by the electrode probe. The same is true to a lesser extent of most normal human muscles, since it is under conditions of minimal contraction that single repetitive action potentials or 'motor units' can best be obtained. Stronger contraction produces a complex record by bringing in adjacent and, as the present studies now suggest, overlapping motor units. Overlapping of anatomical motor units has also been suggested by Jasper & Ballem (1949) to explain the observation that a single unit may continue to be recorded with little change in amplitude while the electrode is moved into the field of a second active motor unit.

Neural pattern in extrinsic ocular muscles

The neuro-anatomical arrangment in the extrinsic ocular muscles is quite distinct from that in any of the other muscles examined. The density of innervation appears to be much greater and is no doubt related to the much higher ratio of nerve fibres to muscle fibres, which in eye muscles has been estimated at 1:5 as compared to 1:50 in the semitendinosus muscle (Bors, 1926) and 1:120 in the soleus muscle (Clark, 1931). Our observations on the mode of innervation of the rabbit's extrinsic ocular muscles agree with the clear description of Hines (1931). In addition, we have shown that the innervation of these muscles in the macaque monkey is similar to that in the rabbit. A distinctive finding is the presence of terminal nerve fibres which give off multiple endings applied in succession along a considerable length of a single muscle fibre. The various types of nerve terminals are described with reference only to their anatomical pattern and not to their functional role. The distinctive nature of the neuro-anatomical arrangements in the extrinsic ocular muscles is of interest, however, in view of the fact that they also differ from other skeletal muscles in the rapidity of their contraction (Cooper & Eccles, 1930), in the higher frequency at which their motor units are capable of discharging (Reid & Whitteridge, 1949) and in their unusual sensitivity to certain drugs (Brown & Harvey, 1941).

SUMMARY

1. Some features of the neural pattern in various striated muscles of the rabbit and macaque monkey have been studied with the aid of intravital methylene blue staining.

2. In the lumbrical muscles of the rabbit the intricate plexiform pattern and the frequent occurrence of axon bifurcation with formation of divergent and recurrent collaterals provide an anatomical means for much commingling and interweaving of the nerve fibres during their distribution within the muscle. As a result of this, one axon entering the muscle may divide to supply end-plates in different parts of the muscle. Conversely, individual end-plates in a given zone of the muscle are frequently derived from intermingling terminal fibres from different parent axons.

3. In other muscles of the rabbit (excluding the extrinsic ocular muscles), although histological examination was not as detailed as for the lumbricals, the neural arrangements appear to be similar.

4. No anatomical evidence has been obtained in support of innervation of single mammalian muscle fibres by more than one axon. In preparations stained with methylene blue, in every instance in which more than one motor end-plate was seen to be related to a single muscle fibre, the end-plates concerned were found to be derived from a common axon.

5. There is evidence that the main features of the neural pattern as described in rabbit muscles may also be found in human muscles. The area of contact and

sampling of electromyographic recording needles is discussed in relation to the intramuscular plexiform arrangement of nerve bundles and the overlap of axonal terminals. The difficulty of recording discrete motor unit action potentials from some muscles is no doubt partly due to the extensive anatomical overlap of axonal terminals from different motor units.

6. The extrinsic ocular muscles show a neural pattern which contrasts with that in most other muscles in that terminal nerve fibres more often run singly and effect less terminal overlap. In addition, a single nerve fibre commonly gives off multiple endings which are deployed along a considerable length of one muscle fibre. These anatomical features may be related to the unique physiological responses exhibited by the ocular muscles.

These investigations were aided by grants from the Medical Research Council of Great Britain and the Rockefeller Foundation. These grants are gratefully acknowledged.

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EXPLANATION OF PLATES

Plate 1

All illustrations are from rabbit muscles.

Figs. 1 and 2. A region of a lumbrical muscle showing the complex pattern of nerve bundles and terminals. Fig. 2 is an outline diagram of the main features of the photomicrograph. The small circle *M* represents the area of contact, 80μ in diameter, of a monopolar microelectrode used in electromyography. The large circle *B* represents the area of contact, 500μ in diameter, of a bipolar concentric electrode made from a No. 23 hypodermic needle. A single beaded fibre, of the morphological type which subserves pain, is indicated by the arrows. Methylene blue, intravenous method; whole muscle preparation. ×110.

Fig. 3. Schematic representation of the gross neural pattern in the extensor digitorum longus muscle as revealed by dissection. Approx. ×2.

Fig. 4. Dissection of sterno-mastoid muscle to show the gross neural pattern. Fine anastomotic nerve bundles which make up an intramuscular nerve plexus are indicated by arrows. Approx. ×3.

Plate 2

All illustrations are from methylene blue stained muscles from rabbit.

Fig. 5. A small zone of lumbrical muscle showing muscle fibres innervated by sprays of terminals from three different nerve bundles—*A*, *B* and *C*. Arrows indicate direction of approach of these bundles. Intravenous method. ×400.

Fig. 6. Sprays of motor endings in crico-thyroid muscle. Note that each spray is made up of multiple nerve fibres which fan out to supply end-plates to a small group of muscle fibres. Local injection method. ×225.

Fig. 7 Intrinsic plantar muscle. Side-view of end-plate showing the sarcolemmal membrane bulging to surround it. Intravenous method. × 900.

Fig. 8. Three axons, one of which gives off a collateral to a motor end-plate supplying a muscle fibre of an intrinsic plantar muscle. Intravenous method. × 900.

Fig. 9. Motor end-plate from crico-thyroid muscle. Local injection method. × 1000.

Fig. 10. Surface view of motor end-plate from crico-thyroid muscle. Local injection methods. × 2900.

PLATE 3

All illustrations are from rabbit muscles.

Fig. 11. Double motor ending lying on one muscle fibre and supplied by twin branches of a single axon. The arrow indicates a single parent axon dividing into daughter axons. Crico-thyroid muscle. Methylene blue, local injection method. × 1400.

Fig. 12. Double end-plate derived from one axonal branch and applied 'in series' to a single muscle fibre. Intrinsic plantar muscle. Methylene blue, intravenous method. × 1500.

Fig. 13. Another form of double end-plate applied to a single muscle fibre. Note difference in diameter of preterminal axons. Intrinsic plantar muscle. Methylene blue, intravenous method. × 1300.

Fig. 14. Two end-plates on the same muscle fibre, as seen in a paraffin section of muscle. The lower end-plate is double. Compare with Fig. 11. Crico-thyroid muscle. Silver impregnation, Bodian protargol method. × 600.

PLATE 4

All illustrations are from extrinsic ocular muscles of macaque monkey,
stained by intravenous methylene blue.

Fig. 15. The single fibre (1) gives off multiple endings, which in this region are all applied to the same muscle fibre. Note fibres (2) and (3), which supply moniliform endings, and the small bundle of preterminal fibres of different sizes (4). The nerve fibres run parallel to the muscle fibres. × 400.

Fig. 16. A terminal nerve fibre of large diameter gives off three endings to the same muscle fibre and a fourth ending to an adjacent muscle fibre. Similar endings are present on the muscle fibres in the lower part of the photograph. × 400.

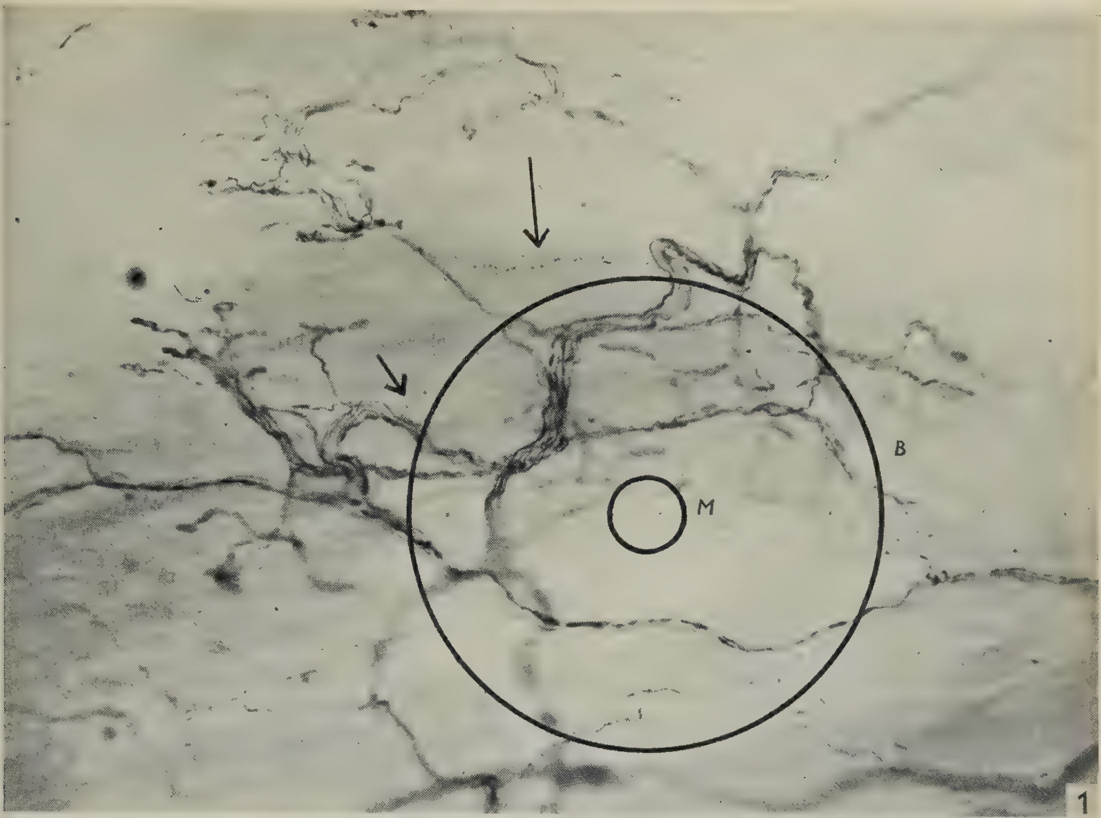
Fig. 17. An isolated fibre giving rise to two endings applied to different muscle fibres. × 400.

Fig. 18. Complex endings near the insertion of the muscle. Note the variation in diameter of the nerve fibres in the small nerve bundle. × 200.

Fig. 19. Complex endings near the insertion of the muscle. × 200.

Fig. 20. The small bundle of nerve fibres supplies endings to several neighbouring muscle fibres. Note the single large fibre which divides into three branches, at lower right-hand corner. × 400.

Fig. 21. A single terminal which resembles motor end-plates found in limb muscles. × 400.



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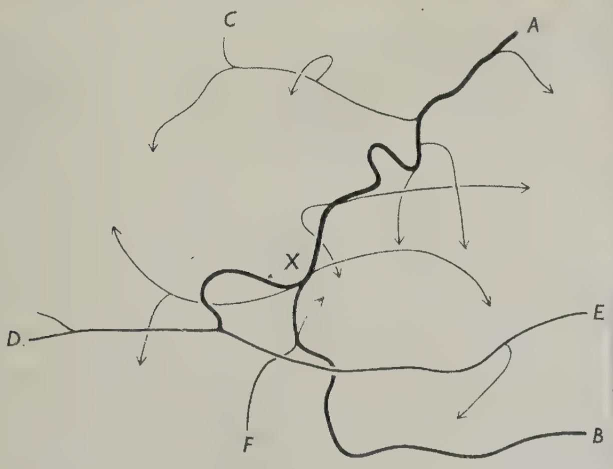

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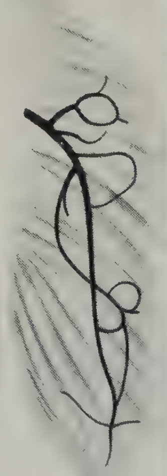

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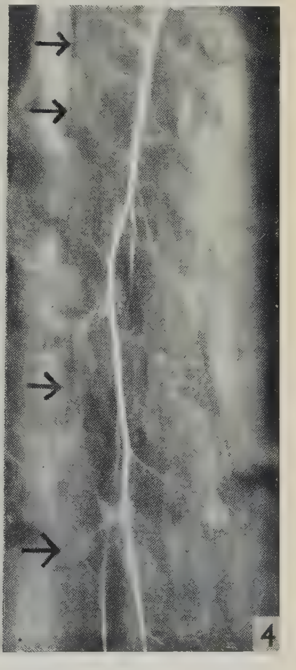

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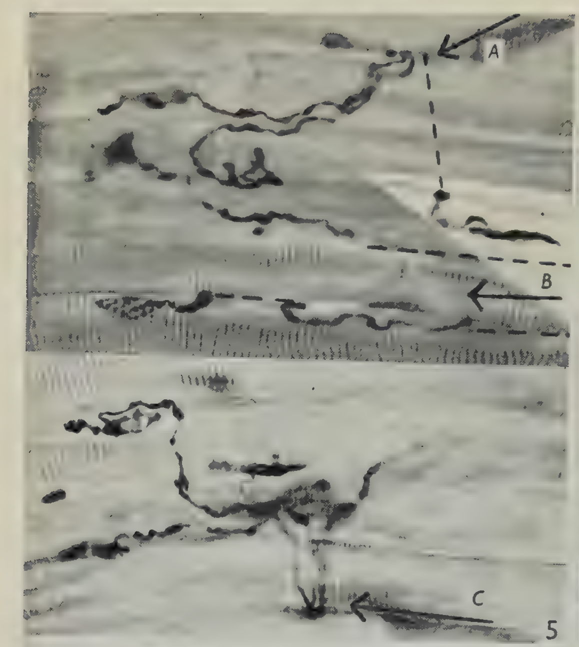
FEINDEL, HINSHAW AND WEDDELL—THE PATTERN OF MOTOR INNERVATION IN MAMMALIAN STRIATED MUSCLE



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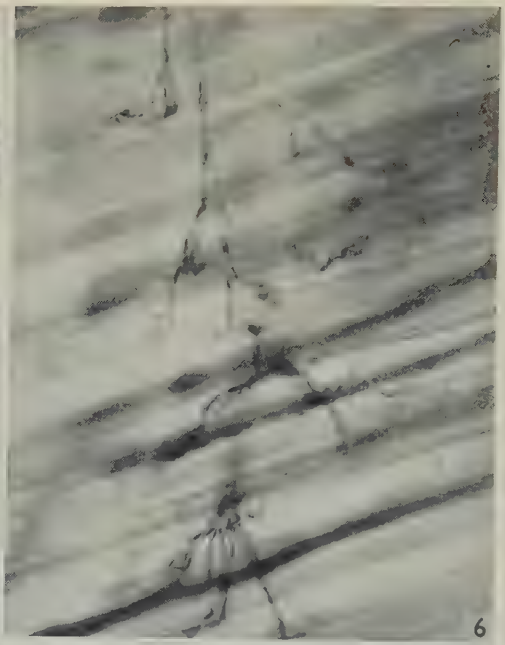

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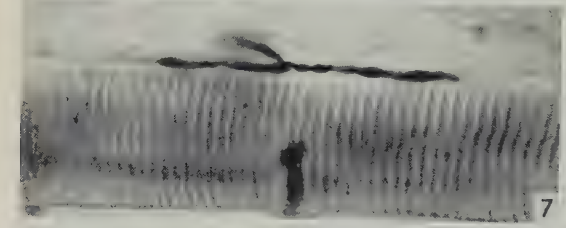

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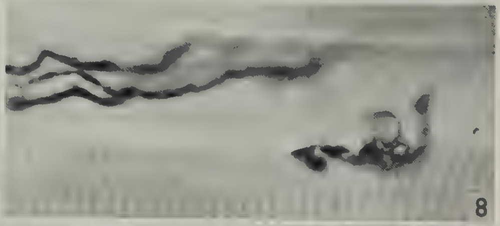

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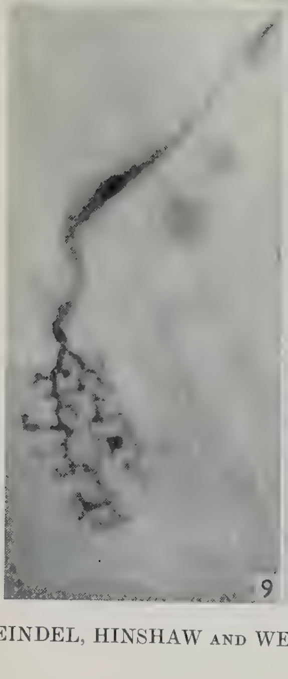

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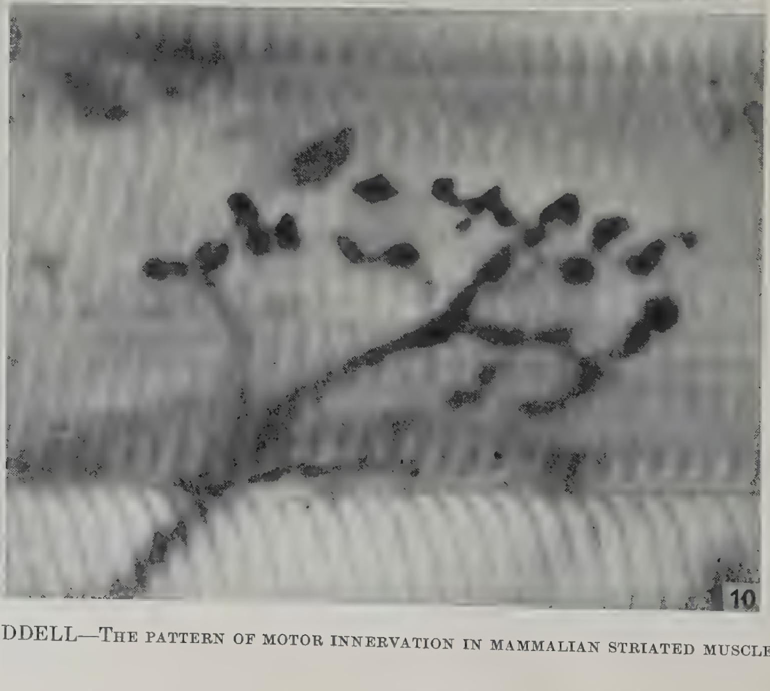

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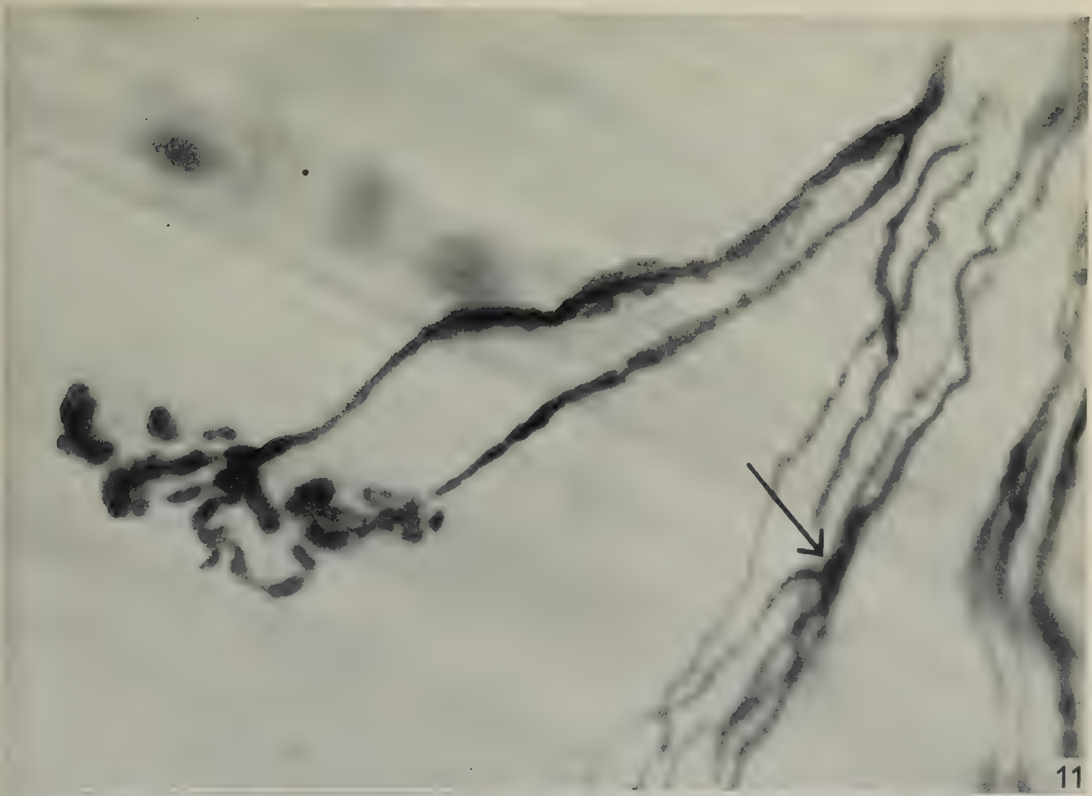
FEINDEL, HINSHAW AND WEDDELL—THE PATTERN OF MOTOR INNERVATION IN MAMMALIAN STRIATED MUSCLE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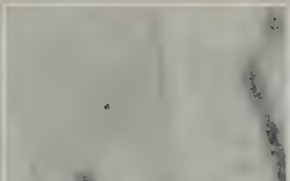
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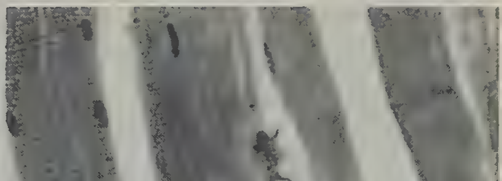
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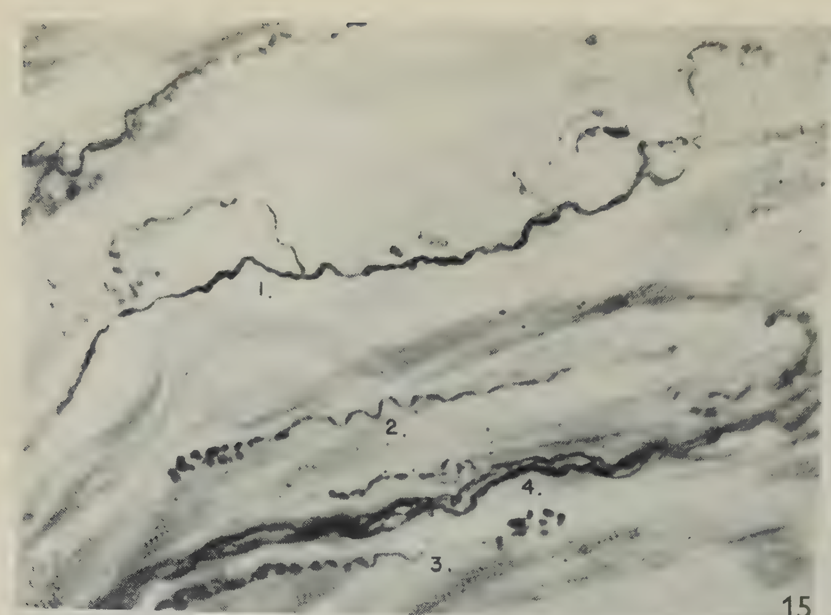

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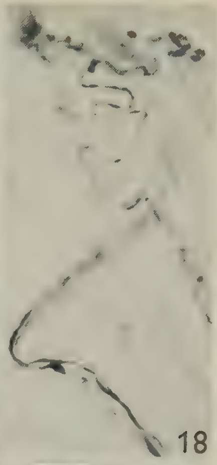

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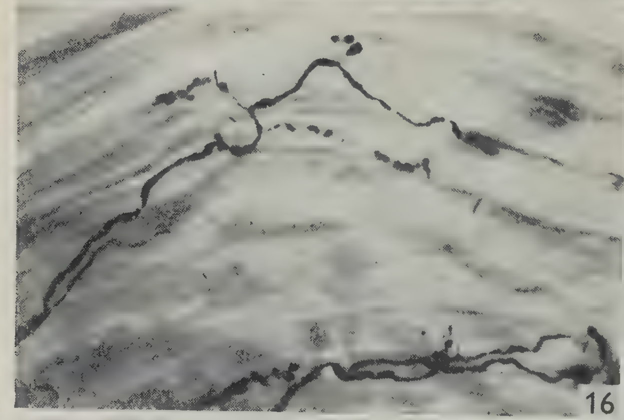

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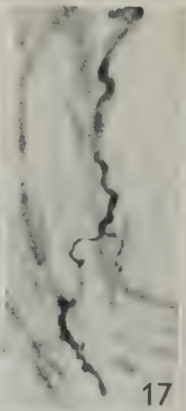

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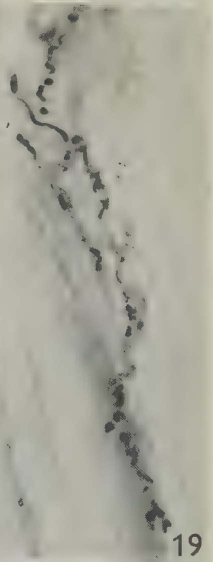

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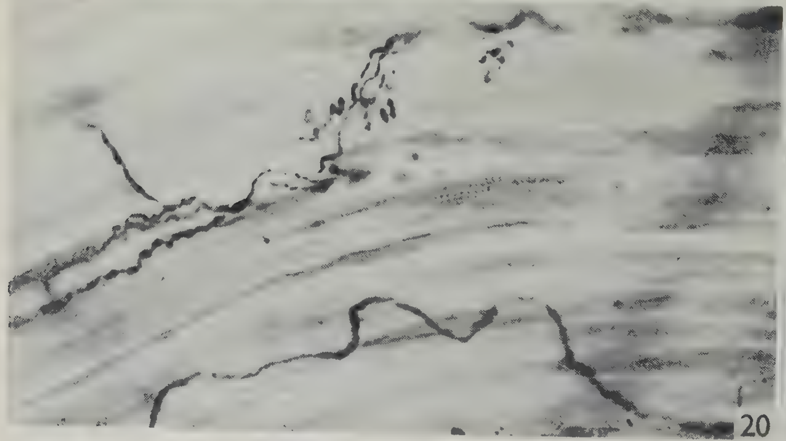

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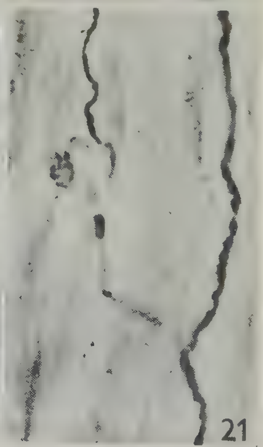

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FEINDEL, HINSHAW AND WEDDELL—THE PATTERN OF MOTOR INNERVATION IN MAMMALIAN STRIATED MUSCLE

THE SILVER STAINING OF MYOEPITHELIAL CELLS, PARTICULARLY IN THE MAMMARY GLAND, AND THEIR RELATION TO THE EJECTION OF MILK

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The existence of myoepithelial cells (basket cells, Korbzelle, Basalzelle) lying between the secretory cells and the basement membrane has long been known in salivary, lacrymal, sweat and mammary glands. They were accurately described and illustrated by German histologists during the nineteenth century.

Kölliker (1850) described long spindle-shaped muscle cells along the outside of sweat ducts, and Henle (1850) referred to a sheath of muscle accompanying the milk ducts deep into the gland substance. Kölliker (1855) could not for certain find muscle cells along milk ducts as in sweat glands, but Henle (1866) quite definitely described and illustrated stellate cells on the basement membrane of the stomach, mammary and salivary glands, which he distinguished from smooth muscle but thought might be nerve cells, which they certainly resemble. Other clear accounts of the mammary myoepithelial cells were given by Langer (1871), Langhans (1873), Moullin (1880), Dreyfuss (1888) and Benda (1893). Two types of myoepithelial cells were distinguished, stellate around the alveoli and spindle-shaped along the outside of the ducts. It was also thought that both types were contractile and aided the escape of the glandular secretions by squeezing the alveoli and widening the ducts. These early accounts of their structure and function have been reviewed by Bertkau (1907), Lenfers (1907), Retterer & Lelièvre (1911), Zimmerman (1898, 1927) and Babkin (1944).

In the case of the mammary glands, considerable interest has recently centred around the mechanism of the 'let down' or ejection of milk which occurs within a minute of the start of milking or suckling, and there is now a great deal of evidence (see reviews by Folley, 1947, 1949) to suggest that this is accomplished by a neurohormonal reflex in response to suckling, whereby a posterior pituitary hormone (possibly oxytocin) causes the mammary myoepithelial cells to contract, and thus move the mass of milk stored within the gland from the alveoli and small ducts to the larger ducts and cisterns, from which it can be easily removed by suckling (Pl. 3, figs. 14, 15). However, for the past 40 years almost the only interest in the mammary myoepithelium has been that of pathologists, who were concerned with its metaplastic activities in mammary tumours (see Hamperl, 1939; Kuzma, 1943), and the only workers considering the normal tissue were apparently unaware of the earlier German work, and confused the cells with smooth muscle (Swanson & Turner, 1941), or considered them capillary adventitial cells (Dieckmann, 1925; Hammond, 1927), so that at the time when Ely & Petersen (1941) put forward the current theory of the ejection mechanism, it is probably true to say that the majority of workers in the field considered that the sparsely distributed smooth muscle was the effector tissue. The balance was ably restored by Richardson (1949), who, aware of the true nature of the myoepithelial cells, and the difficulty of staining them by conventional methods, devised a silver staining method which dramatically demonstrated the extensive network of cells around all the alveoli and ducts of the mammary gland. He also again clearly differentiated the cells from smooth muscle, and produced circumstantial evidence for their contractility and participation in the 'let down' reflex.

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* Work mostly carried out in the Department of Physiology, University of Edinburgh.

At a time (May, 1948) when I was unaware of Richardson's work on the goat, or that Zimmermann (1898) had previously used a chrome silver staining method, the myoepithelium of the mammary gland of a lactating cat was accidentally stained with silver when trying to stain nerve endings. The purpose of this paper is to provide independent confirmation of the work of Richardson, by showing the existence of these silver staining cells (which can be identified as the myoepithelium) in the mammary, salivary and sweat glands of other species, and to discuss their relationship to the ejection of milk.

METHODS

The lactating mammary glands of twenty-six cats, five dogs, one rabbit, one rat, one goat and two humans were used. The animal tissues were taken and fixed (frequently by perfusion) before death from anaesthetized animals used in the experiments of Linzell (1950). The human material was obtained 10 and 24 hr. after death and fixed immediately. The submaxillary and salivary glands and pancreas of a day old kitten and of two cats were also used.

The following fixatives were used: 10 % formalin in N saline (neutral and acidified); 15 % neutral formalin and 2 % ammonium bromide; 25 % chloral in 50 % alcohol; formalin 1 part and saturated mercuric chloride 9 parts; Carnoy (absolute alcohol 6 parts, glacial acetic acid 1 part and chloroform 3 parts); Helly's fluid; Bouin; Weber's (1944) fluid and acetic-formal-alcohol (glacial acetic acid 5 % and formalin 5 % in 80 % alcohol).

Frozen and paraffin embedded (dehydrated and cleared with isopropyl alcohol, ethyl alcohol and chloroform) sections were stained, in many cases from the same piece of tissue. A routine thickness of 25 μ was generally employed. No advantages were found in thicker sections (up to 100 μ) as recommended by Richardson (1949), and thinner sections were less suitable because whole myoepithelial cells were less frequently included in a section.

The silver-staining methods of Gros-Bielschowsky (see McClung, 1950), Rogers (1931), Nonidez (1939), Holmes (1942, 1943), Silver (1942), Richardson (1949) and Romanes (1950) were tried.

RESULTS

The findings of Richardson (1949) were confirmed. It was possible to stain the myoepithelial cells with silver in the mammary glands (Pl. 1, figs 1–6; Pl. 2, fig. 7, 9, 11; Pl. 3, figs. 12, 13), in the sweat glands of the mammary skin (Pl. 2, fig. 8), and in the submaxillary salivary gland (Pl. 2, fig. 10), but nothing resembling these cells was seen in the pancreas.

Of the fixatives and staining methods tried, success was only obtained with tissues fixed in Carnoy, acetic-formal-alcohol or formalin (acidified) and with the staining methods of Romanes and Richardson.

Richardson's method was not used as extensively as that of Romanes and failed to stain the myoepithelium of the goat udder, fixed in Webers' fluid (not perfused as Richardson recommended) and also failed on dog and cat tissue fixed by perfusion with Webers' fluid. However, it did stain the myoepithelial cells and other cell nuclei of four pieces of cat tissue, two fixed in acetic-formal-alcohol (one by perfusion), one in acidified formalin, and one in Carnoy's fluid (by perfusion).

The technique of Romanes (1950), using a weak solution of colloidal silver chloride at pH 8·0 (16 hr. at 56° C. in the dark), gave better but not completely reliable results. After staining, the sections were developed in hydroquinone and sodium sulphite solution, but the toning in gold chloride was omitted as it was often found to increase the density of background staining. With this method only Carnoy and acetic-formal-alcohol fixatives gave positive results, and in the case of the former, immersion in 10 % formalin overnight before cutting frozen sections or embedding was found to be essential. The addition of 5 % formalin to the Carnoy did not have the same effect.

In addition to the myoepithelial cells (brown to black), smooth muscle (golden brown) and nerves (black) were also stained (Pl. 1, figs. 1, 3), whilst the background was either unstained or showed only the outlines of secretory cells, particularly their nuclei and nucleoli. Failure to obtain this specific staining of myoepithelial cells, smooth muscle and nerve was encountered, in which case either the result was a faint general brown colour or collagen and reticular fibres were stained (Pl. 1, fig. 2). The immersion of the sections in 5 % potassium dichromate for a few hours before staining was sometimes found to alter completely the pattern of staining from the latter to the former (particularly in acetic-formal-alcohol fixed tissues), but paradoxically also sometimes changed it from one showing myoepithelium, muscle and nerve to that showing collagen and reticulin (see Table 1). Many variations were made in unsuccessful attempts to find a reliable method of ensuring the specific staining required along the lines suggested by Kubie & Davidson (1928), Foot (1929*a*, *b*, *c*), Foley (1939), Landau (1940), Silver (1942), Holmes (1942) and Weber (1944), but these will not be given, as it is not the purpose of this paper to describe a method. The reliability of the Romanes method as used may be judged from Table 1.

As noted by Richardson the capillaries were also occasionally stained in parts of otherwise satisfactorily stained sections (Pl. 2, fig. 11).

There can be no doubt that the silver-stained cells seen in the tissues studied are the myoepithelial cells, as previously described and illustrated by the early German histologists. The stellate variety lie as a complicated irregular network around the alveoli at the base of the secretory cells (Pl. 1, figs. 1, 5, 6; Pl. 2, figs. 7, 9), and sweep uninterruptedly over onto the ducts where they become spindle-shaped (Pl. 2, fig. 9) and are found lying longitudinally outside the normal two-cell lining (Pl. 1, fig. 4; Pl. 2, fig. 9), right up to the cisterns and teat ducts. The silver method used does not stain the basement membrane, so that its relationship to the cells cannot be seen in these preparations, but in this connection it is interesting to note

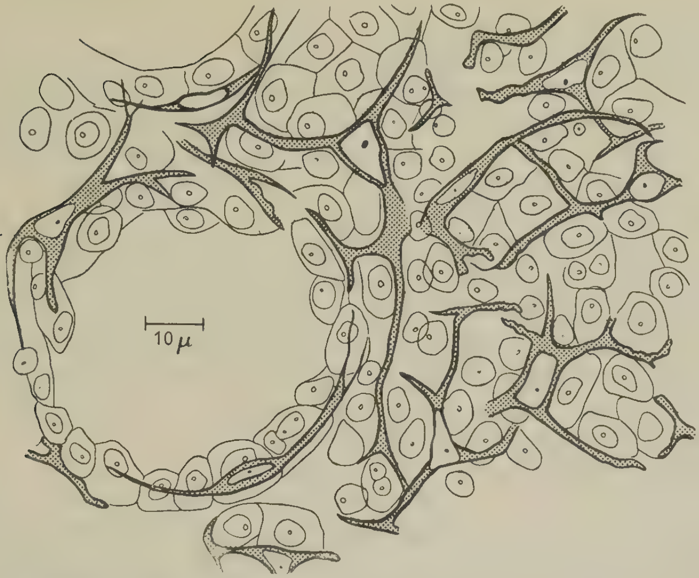
Table 1. *Results of staining lactating mammary glands by Romanes's method for the demonstration of myoepithelial cells*

No.	Species	Frozen sections: No pretreatment	Frozen sections: $K_2Cr_2O_7$ pretreatment	Paraffin sections: No pretreatment	Paraffin sections: $K_2Cr_2O_7$ pretreatment
		Carnoy fixed tissues followed by formalin			
27	Cat	.	.	+ +	−
30	Cat	.	.	+ +	+ +
32	Cat	+ +	.	−	+ +
39	Dog	+ −	+ +	+ −	−
60	Cat	+ −	+	+ −	−
61	Cat	+ +	.	.	.
62	Cat	+ +	+ +	+ +	+ +
69	Cat	+ −	.	.	.
74	Human	+ −	+	+	+ −
75	Rabbit	−	+ −	+ −	+
76	Cat	+ +	.	.	.
79	Human	−	+ +	+ +	−
81	Dog	+ +	+ +	+ +	+ +
86	Dog	+ −	−	− ?	+ +
87	Rat	+	−	+ − ?	−
90	Goat	+	+	.	.
		Acetic-formal-alcohol fixed tissues			
50	Cat	+ +	.	+ +	+ +
52	Cat	+ +	.	+ +	+ +
59	Dog	−	+ −	.	.
69	Cat	+ −	+ −	+ +	+
67	Cat	−	−	−	−
71	Cat	−	−	+	+
72	Cat	−	+ −	+ −	+ +
73	Cat	−	+ +	−	−
74	Human	−	+	+ −	−
75	Rabbit	−	+	−	+
78	Cat	−	+	+	+
79	Human	−	+ −	.	.

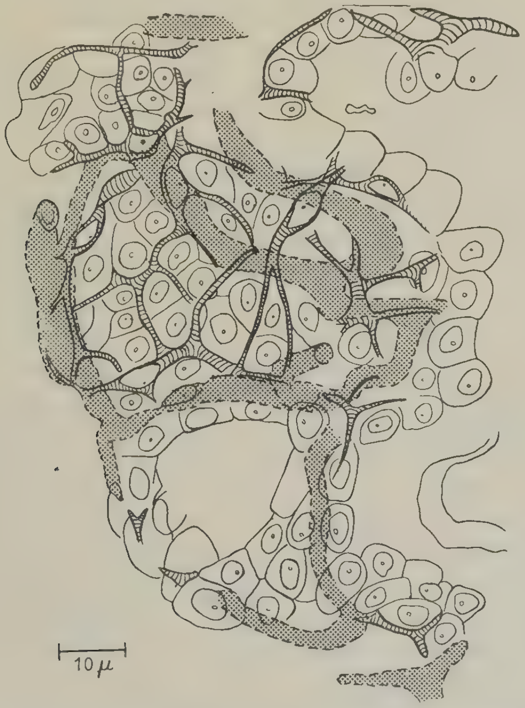
+ +	Myoepithelial cells distinctively stained on faint background.
+	Positive, but not so easy to see, since background stained more.
+ −	Cells faintly stained.
−	Negative. Myoepithelium unstained.

that pathologists (Hamperl, 1939; Kuzma, 1943) have studied the myoepithelium in an indirect manner by staining the basement membrane with silver. Their studies confirm that the myoepithelium lies between the secretory cells and the basement membrane. However, it was not until silver nerve stains were applied to the mammary gland that it was accidentally and independently discovered that the myoepithelium itself could be stained with silver. It should also be pointed out here that it is misleading to refer to the outer cell layers of the ducts as myoepithelium (Dempsey, Bunting & Wislocki, 1947), because the myoepithelial cells in fact lie outside as a third layer (Pl. 2, fig. 9).

The spindle-shaped cells lying along the periphery of the ducts are clearly separate, but the complicated network of stellate cells over the alveoli often appears syncytial. However, in these cases careful drawings have been made at the highest magnification (see Elias, 1950), following out the finest ramifications. Although cell processes do sometimes meet and often cross over, no evidence that



Text-fig. 1. Free-hand drawing showing that the mammary myoepithelial cells, as seen in section and from the surface, do not form a true syncytium.



Text-fig. 2. Free-hand drawing of field photographed in fig. 11, Pl. 3, showing relationship between myoepithelial cells and capillaries. The myoepithelial cell bodies are all very close to capillaries.

the myoepithelium is a true syncytium was obtained, and the opinion was formed that it is probably not (Text-figs. 1, 2).

The capillaries lie very close to the myoepithelial cells, and in some cases appear to be joined to some of the myoepithelial processes (Pl. 2, fig. 11). Careful examination in the same way, however, shows there is no direct union (Text-fig. 2).

Although the silver method stains the finest nerve endings accompanying arterioles, no nerves have ever been seen to enter either the secretory cells or the myoepithelial cells in this study.

Richardson's finding that the cells differ in appearance in distended and empty glands, being long and drawn out in the former and short and squat in the latter has been confirmed. The cells in a distended specimen in fig. 12 (Pl. 3) should be compared with those in fig. 13 (Pl. 3), in which the gland was thoroughly emptied by oxytocin injected intravenously before fixing. The cells around completely collapsed alveoli appear much broader and it is often possible to make out a distinctly fibrillar internal structure in this state (Pl. 2, fig. 7).

DISCUSSION

The discovery that silver can, under certain circumstances, be used to obtain a fairly selective staining of myoepithelial cells, has, in the case of the mammary gland, helped to clear up a number of points, which have previously caused confusion, as to the peripheral mechanism of the 'letting down' or ejection of milk. The following facts are offered in support of the view that the mammary myoepithelium is the sole effector tissue concerned in the process:

(*a*) The silver staining methods reveal the extreme abundance of the myoepithelial tissue and emphasise that it is ideally arranged around the alveoli and along the ducts, for squeezing the milk out of the acini and small ducts into the large ducts and cisterns (Pl. 3, figs. 14, 15).

(*b*) The new method which also selectively stains smooth muscle and nerves, reveals that in the species studied, there is no interalveolar smooth muscle and that the myoepithelial cells in the mammary gland are not innervated. The effector tissue could not therefore be smooth muscle as was suggested for the cow by Swanson & Turner (1941), and the absence of innervation fits in with the finding that ejection can be obtained in a denervated (Ely & Patersen, 1941) or isolated perfused mammary gland (Petersen, Shaw & Visscher, 1941; Peeters & Massart, 1947; Linzell, 1950) and rules still further against Gaines's (1915) theory of a purely nervous reflex.

(*c*) Hammond (1936) postulated that the 'let down' phenomenon was due to a vascular erection process, similar to the penis, since early German anatomists had described a network of veins in the cow's teat resembling cavernous tissue (see Riederer, 1903; Rubeli, 1916; Zietschmann, 1917). Subsequent histological studies of injected tissues (Dabelow, 1933; Linzell, to be published) have failed to demonstrate any vascular arrangement that could 'erect' the mammary gland itself, and stimulation of the mammary vasomotor nerves or mechanical compression of the venous outflow have not been found to cause the ejection of milk (Linzell, 1950). However, the very close proximity of the myoepithelial cells to the capillary network around alveoli and ducts, means that any circulating oxytocin would reach them quickly and would probably affect all the myoepithelial cells almost simultaneously.

(*d*) The myoepithelial cells stain when smooth muscle stains and have what appear to be myofibrils inside them. The distinct shortening and thickening of the cells occurring when the alveoli empty, naturally in response to milking (Richardson, 1949) or artificially in response to oxytocin (Pl. 3, fig. 12, 13), strongly suggests that their role in the process is active and not passive, when folding of the long cell processes would be expected.

(*e*) The mammary myoepithelial cells are much longer and more extensive than those in the sweat glands or salivary glands, in which secretion is mainly controlled by secretory nerves. Milk secretion is not controlled in this way, and is believed to be a more or less continuous process, depending upon intra-mammary pressure, and hormonal and metabolic factors. Some active muscular process is therefore essential for removing the stored milk, and it is significant that the myoepithelium is so extensive in the mammary gland.

SUMMARY

The findings of Richardson (1949) that the myoepithelial cells of the mammary gland of the goat can be selectively stained with silver has been independently confirmed for other species and extended to the same cells in the sweat glands and the submaxillary salivary glands.

The appearance of the myoepithelium in silver stained sections is described and evidence discussed in favour of the view that this tissue in the mammary gland is responsible for the phenomenon of the 'letting down' or ejection of milk in response to suckling or milking.

It is with very great pleasure that I acknowledge the technical assistance of Mr A. Marshall, M.B.E. The section in which the silver stained myoepithelial cells were first noticed was prepared by him, and he gave freely of his considerable experience in helping me to follow up this observation. I am grateful to Dr G. J. Romanes for letting me use his staining method sometime before it was published and to Dr A. W. B. Cunningham for obtaining the human tissues for me.

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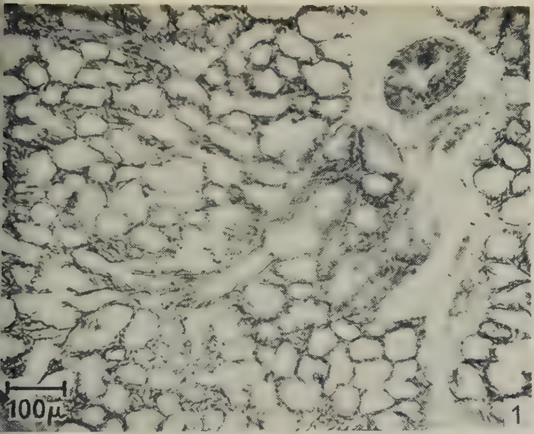
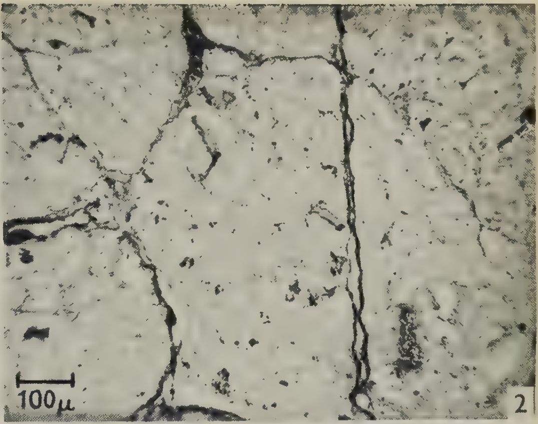
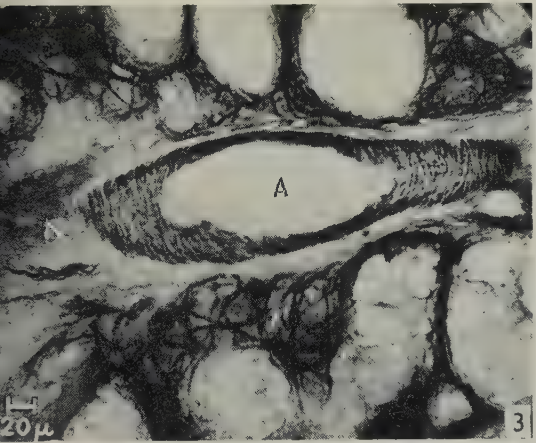
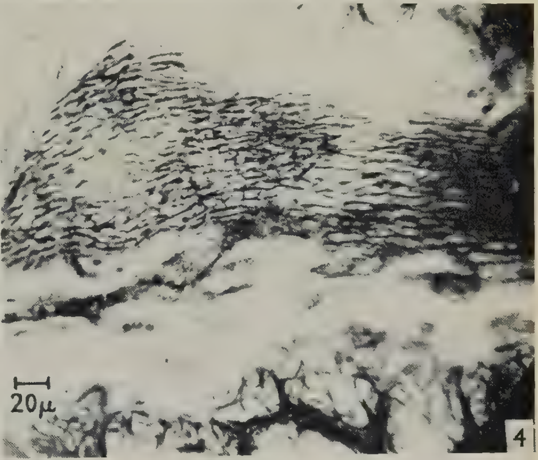
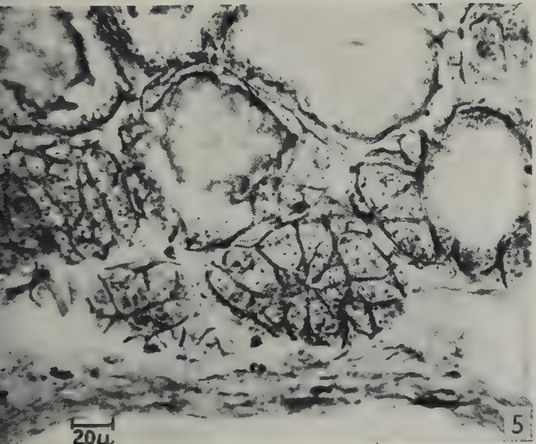
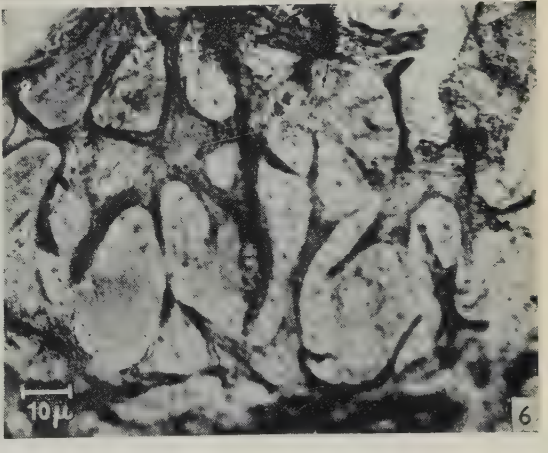




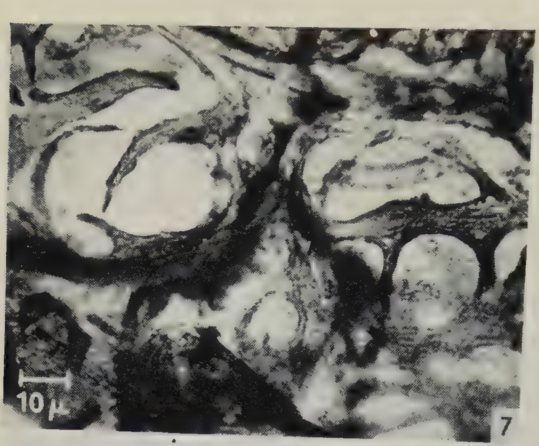








LINZELL—SILVER STAINING OF MYOEPITHELIAL CELLS



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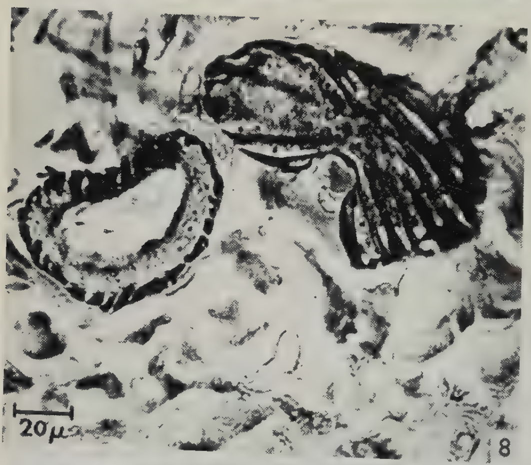

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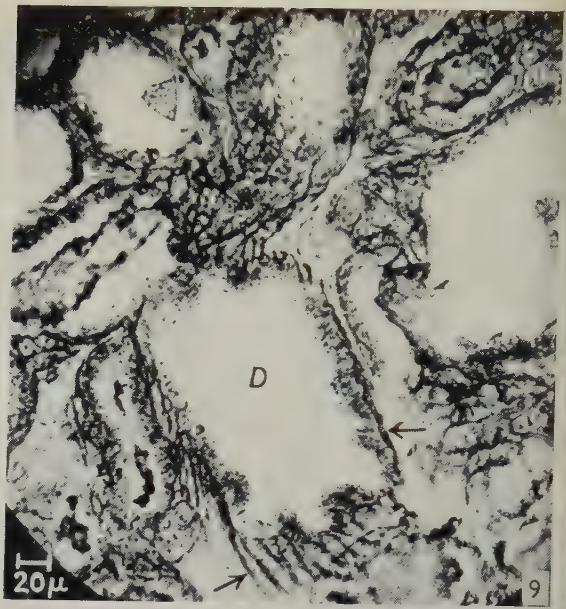

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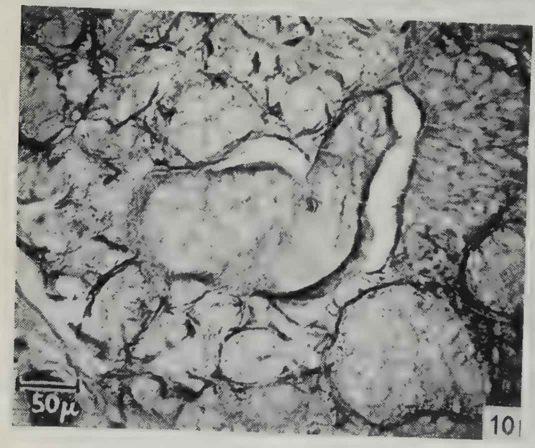

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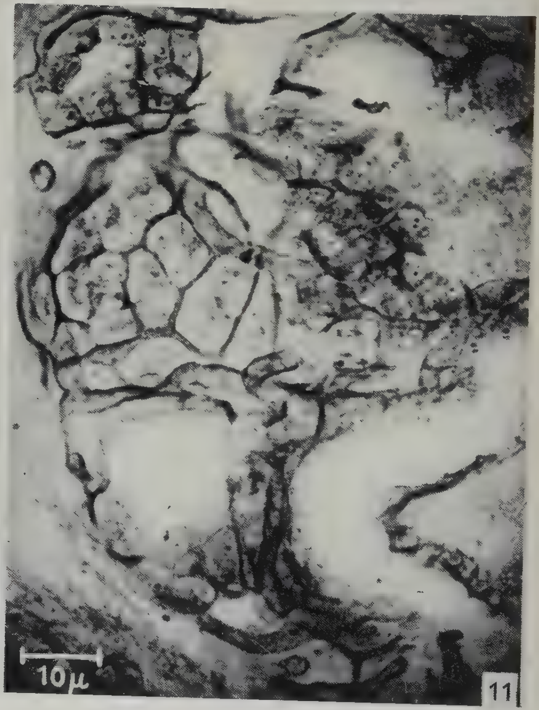

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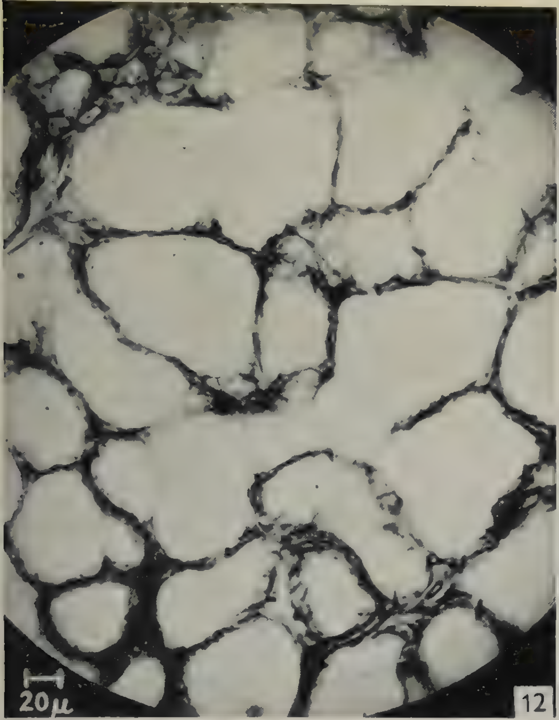
LINZELL—Silver staining of myoepithelial cells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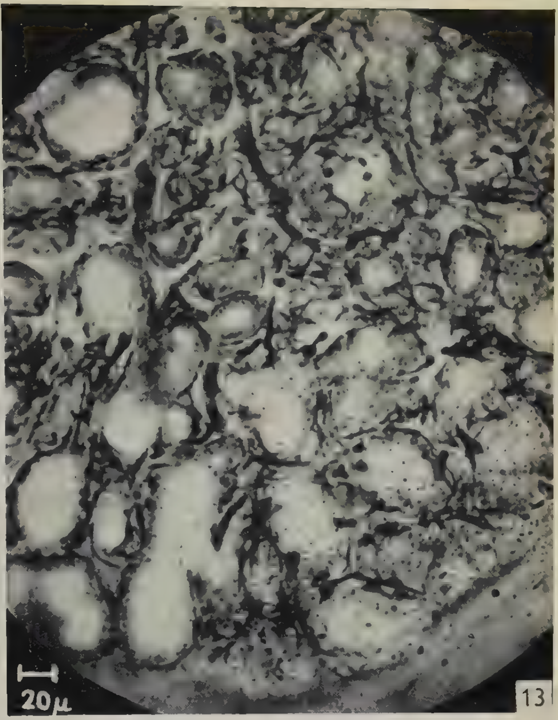
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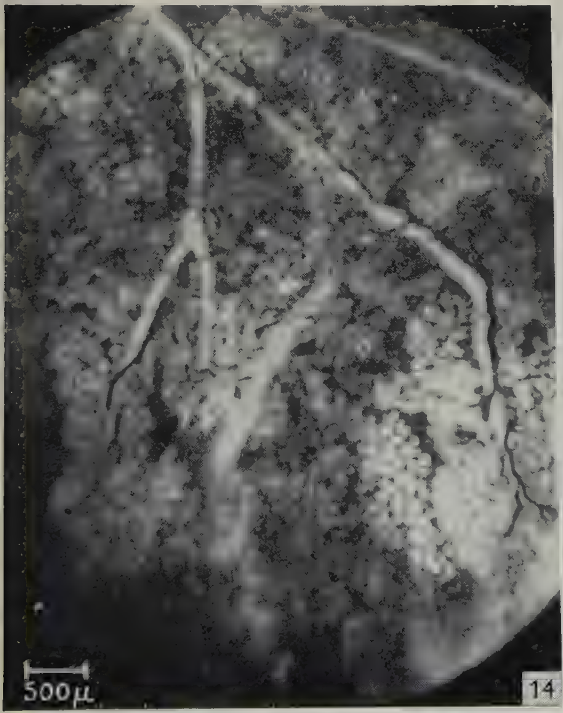
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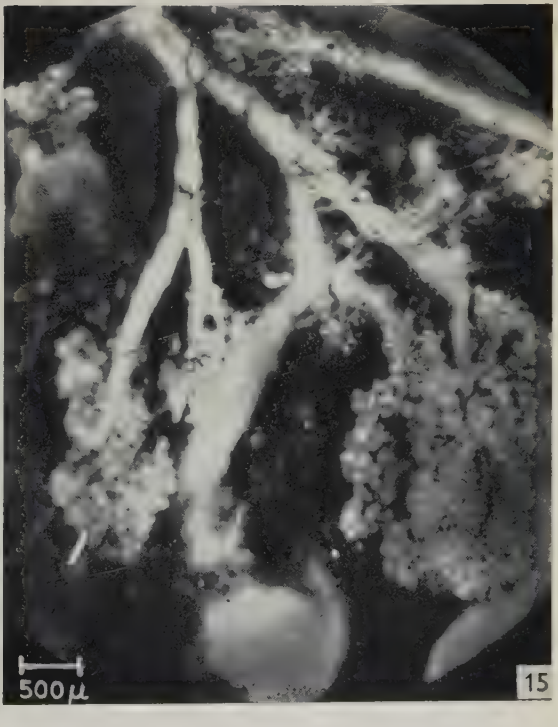
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LINZELL—SILVER STAINING OF MYOEPITHELIAL CELLS

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EXPLANATION OF PLATES

Photomicrographs of 25 μ sections stained by Romanes's method. All lactating mammary tissue, except figs. 8 and 10.

Plate 1

Fig. 1. General low power view showing abundance of myoepithelium. Background unstained. Tissue no. 32. Cat. Carnoy. Frozen section.

Fig. 2. General low power view showing different appearance when collagen and reticulin stained. This could not be confused with the specific myoepithelial staining in fig. 1. Tissue no. 52. Cat. Acetic-formal-alcohol. Frozen section.

Fig. 3. Simultaneous staining of smooth muscle of an artery (A) and vasomotor nerves in nerve bundle (N) as well as myoepithelium. Tissue no. 32. Cat. Carnoy. Frozen section.

Fig. 4. Same section as fig. 3, showing longitudinal myoepithelium on surface of large interlobar duct.

Fig. 5. Network of stellate myoepithelial cells over surface of alveoli. Tissue no. 52. Cat. Acetic-formal-alcohol. Frozen section.

Fig. 6. Stellate myoepithelial cells from a dog. Tissue no. 81. Carnoy. Paraffin section.

Plate 2

Fig. 7. Myoepithelial cells in an empty mammary gland showing a fibrillar internal structure. Tissue no. 76. Cat. Carnoy. Frozen section.

Fig. 8. Myoepithelial cells lying outside cells of sweat gland in dermis over mammary gland. Tissue no. 27. Cat. Carnoy. Paraffin section.

Fig. 9. Myoepithelium (marked by arrows) lying outside two-cell layered wall of milk duct (D). Tissue no. 52. Cat. Acetic-formal-alcohol. Paraffin section.

Fig. 10. Submaxillary salivary gland of cat showing smaller, less numerous myoepithelial cells. Carnoy. Paraffin section.

Fig. 11. Myoepithelial cells and capillaries stained by silver. Tissue no. 50. Cat. Acetic-formal-alcohol. Paraffin section. A freehand drawing of this field is shown in Text-fig. 2.

Plate 3

Fig. 12. Section of gland in distended condition, showing long drawn out cells. Tissue no. 60. Cat. Carnoy. Frozen section.

Fig. 13. Section of gland from same animal emptied of milk with the aid of oxytocin, showing squat, globular myoepithelial cells.

Photomicrographs of the living mammary gland of an anaesthetised lactating mouse with blood and nerve supply intact, 10 hr. since last suckled.

Fig. 14. Normal appearance of distended gland showing evenly distributed alveoli as white dots and white milk filled ducts.

Fig. 15. Same field 2 minutes after applying 0·01 unit of oxytocin (Pitocin, P.D. and Co.). Note that the majority of alveoli have been emptied of milk and can no longer be seen, whilst the ducts have become greatly distended. Two most superficial lobes have not been emptied, probably because the milk could not escape as the ducts were distended to capacity. Some milk has spilled out from an injury onto the surface of the gland.

AN EXPERIMENTAL STUDY OF HIPPOCAMPAL AFFERENT PATHWAYS FROM PREFRONTAL AND CINGULATE AREAS IN THE MONKEY

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INTRODUCTION

The role of the cingulum bundle as a source of afferent fibres to the hippocampus has received considerable attention since Papez (1937) suggested that the cingulate cortex and hippocampus form part of a mechanism concerned in emotional control. He postulated a circular pathway from the hippocampus via the fornix to the mamillary body and thence by further relays to the anterior nucleus of the thalamus and the cingulate cortex. He envisaged the completion of this pathway by fibres running posteriorly in the cingulum bundle from the cingulate cortex to the hippocampus. Cajal (1911) had noted such fibres in the cingulum of rabbits and mice in Golgi preparations, and observed that the cingulum increases in size antero-posteriorly.

More recently, experimental investigations of the hippocampal connexions of the cingulate cortex in animals and man have suggested the possibility of generic differences in the various contributions of the mid-line cortex to the cingulum bundle. Meyer, Beck & McLardy (1947) traced gliosis in human leucotomy material not further than 3 cm. behind the cut. Beck (1950), after further study, followed the gliosis to the level of the splenium, but could not demonstrate degeneration in the hippocampal region. Glees, Cole, Whitty & Cairns (1950) found that in the monkey, after lesions in area 24 with injury to the cingulum, Marchi granules could be traced into the retrosplenial area. They suggest that the cingulum is not so intimately related to the gyrus cinguli as some have thought. In the rabbit, ablation of the anterior cingulate and adjacent frontal cortex produces severe degeneration in the cingulum in fibres terminating in the hippocampal presubiculum (Adey, 1951). M. Meyer (1950) also noted terminal degeneration in the presubiculum of the macaque after ablation of area 24.

The present study in the monkey has aimed at the determination of the extent of the connexions of different parts of the cingulate cortex with the hippocampus through the cingulum bundle, and to determine whether certain cortical areas on the medial aspect of the frontal lobe, particularly those undercut or ablated by leucotomy procedures such as the transorbital approach of Freeman (1948), might contribute significantly to the cingulum. The satisfactory results reported after this operation, and the contrasting absence of lasting improvement after a simple removal of anterior cingulate cortex (Glees *et al.* 1950), suggested that the therapeutic value of the medial leucotomy of Freeman might lie in its interruption of fibres contributed to the cingulum from granular frontal cortex (areas 9 and 10). Mere ablation of anterior cingulate cortex would not necessarily interrupt these fibres running in the subjacent white matter.

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MATERIAL AND METHODS

Cortical ablations have been performed in five green monkeys (*Cercopithecus sabaeus*). All operations were performed under intraperitoneal nembutal anaesthesia with full aseptic technique. In all but one experiment the cortical area was first undercut with an ophthalmic knife and then removed by careful suction. In this way damage to adjacent cortical areas was minimized. Particular care was exercised in retracting the opposed medial hemispheric walls to avoid damage to the overlying parts of the frontal and parietal lobes. The animals were allowed to survive 5–7 days after operation, and were then perfused under nembutal anaesthesia with normal saline and the brain fixed *in situ* with 10 % formalin in normal saline. After removal, the brain was fixed for a further 10 days in 10 % formalin in normal saline.

In two cases the lesion was mainly in the anterior cingulate cortex, in one in the posterior cingulate area, and in two cases it involved mainly granular frontal cortex. The brains were examined for degenerating fibres and terminals by staining with a modification of the Glees (1946) ammoniacal silver method. For this method frozen sections were cut at 10–15 μ, and a few thicker sections were also cut at each level at 25–30 μ for staining with Nissl and Weil techniques. Serial sections of normal material stained by Nissl and Weigert methods were available for comparison.

The following is a summary of the modifications of the Glees method used in this study.

(*a*) The frozen sections are washed in distilled water, and then placed in 50 % ammoniacal alcohol for 48 hr. (12 drops of concentrated ammonia solution in each 100 c.c. alcohol used).

(*b*) They are transferred first to 10 % alcohol and then to distilled water. This should be done in deep vessels and the sections prevented from rising to the surface.

(*c*) The sections are placed in 10 % aqueous silver nitrate solution and stored in the dark for 24–48 hr. The sections should not be allowed to become darker than orange-brown before proceeding to the next stage.

(*d*) Final staining: (i) The sections are transferred rapidly through each of three dishes containing 10 % formalin in tap water. A white precipitate pours from the sections which are therefore kept moving to avoid precipitation on the surface of the section. (ii) Place in an ammoniacal alcoholic silver bath for 45–90 sec. 20 % aqueous silver nitrate solution, 3 parts; 96 % alcohol, 2 parts. Add concentrated ammonia drop by drop until the precipitate which forms just redissolves. (iii) Transfer rapidly through each of three dishes of 10 % formalin in tap water as in (i). The sections now turn a dark brown. If the sections are too dark, more ammonia should be added to the ammoniacal silver bath. If too pale, either cautiously add more silver nitrate solution to the bath, or the section may be passed through the silver bath a second time.

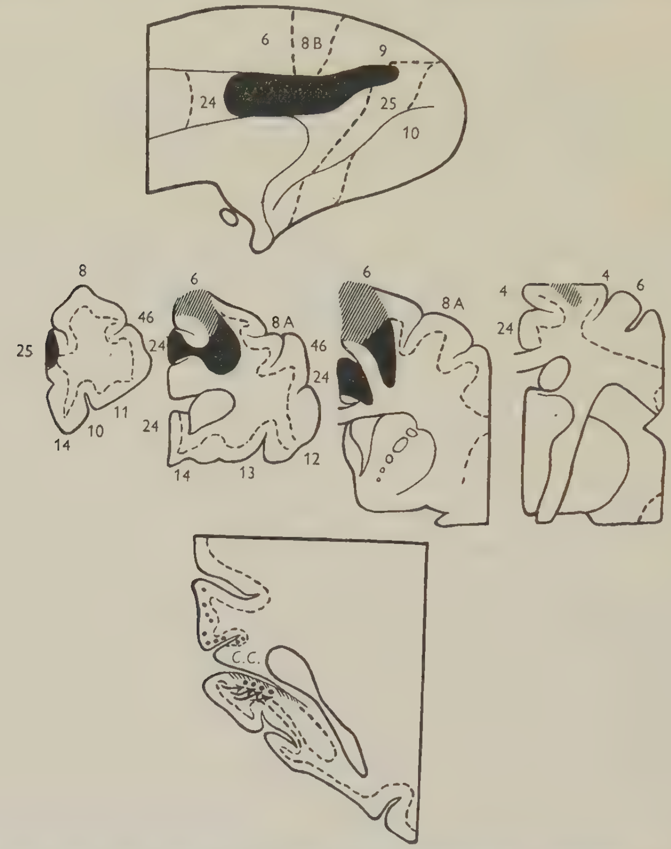
(*e*) Dehydrate in alcohol, transfer to carbol-xylol and mount.

EXPERIMENTAL RESULTS

In the description of the lesions, Walker's classification (1940) of the cortical areas of the frontal lobe in the monkey has been followed, while Brodmann's (1909) and von Economo's (1929) subdivisions of the posterior limbic areas and the hippocampal formation have been used where indicated.

A. *Ablation of the anterior cingulate gyrus*

Case 1 (M 81). This animal was killed 6 days after operation. The lesion (Text-fig. 1) was effected by diathermy and lies mainly within area 24, extending slightly into area 25. It extends to within 5 mm. of the caudal margin of area 24, leaving the posterior cingulate area undamaged. The cingulum itself is probably severed. Coagulation of a branch of the anterior cerebral artery has resulted in softening of tissue in the medial part of area 6.



Text-fig. 1. Anterior cingulate ablation, case 1 (M 81), showing the extent of the lesion. Areas secondarily involved by vascular injury are indicated by hatching. As in the following text-figures, the extent of the primary lesion is indicated (in black) on the surface of the brain and in transverse sections. Below, the site of degenerating fibres and terminals is shown diagrammatically. Degenerating fibres are indicated by arrows, and terminal degeneration by coarse dots. *C.C.* = corpus callosum.

Microscopic examination of silver impregnated transverse sections at the level of the splenium of the corpus callosum (Text-fig. 1) shows fibre degeneration in the cortex of the posterior cingulate region. A careful examination of serial Nissl material of the cingulate cortex in the monkey at this level shows that it is divisible into a number of horizontal strips. Dorsally, extending into the cingulate sulcus is a narrow portion named by Brodmann area 31, the *area cingularis posterior dorsalis*, and by Economo in the human brain area LC 1. Bailey, Bonin, Davis, Garol, McCulloch, Roseman & Silveira (1944) have shown this area to be a suppressor strip. The major part of the free surface of the cingulate gyrus is formed by area 23 of Brodmann, area LC 2 of Economo, the *area cingularis posterior ventralis*. A narrow band of agranular cortex occupies the lower edge of the free surface of the cingulate gyrus (area 30, area LD, the agranular retrosplenial region). This extends laterally into the granular retrosplenial regions (area 29, areas LE 1 and LE 2) in the superior lip of the callosal sulcus (Text-fig. 6).

In this experiment there are scanty degenerating fibres in the cortex of areas 23, 30 and 29, terminating in some instances in the superficial layers. There is some terminal degeneration among the closely packed cells of the indusium griseum, but the immediately adjacent supracallosal striae appear normal. The state of the fibres in the cingulum bundle itself is difficult to determine as they have been sectioned nearly transversely.

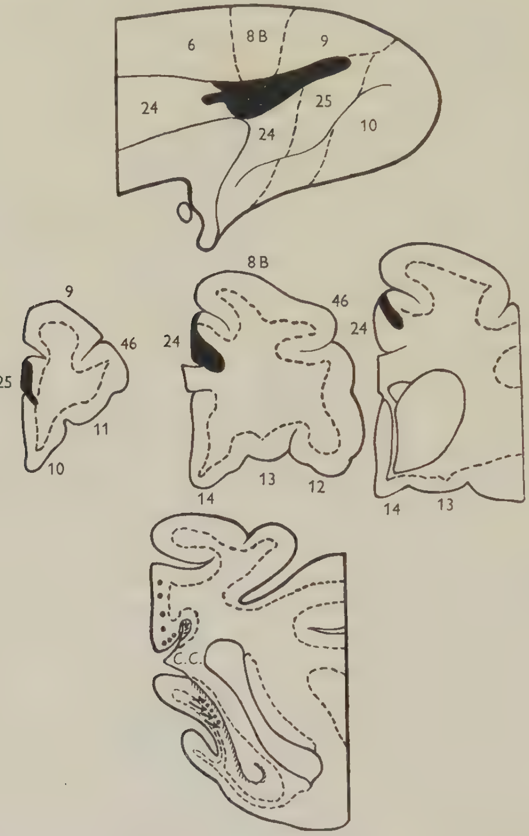
Ventral to the corpus callosum, the same coronal sections show a gradual transition as the cortex is traced on to the ventral aspect of the corpus callosum (Text-fig. 6). From the retrosplenial cortex on the free surface of the hemisphere, there is a smoothly sweeping arc of buried cortex which narrows rapidly to a thin lamina ending ventrally in the layer of hippocampal pyramidal cells. The upper part of this narrow cortical strip has densely grouped small cells in the second and third layers and forms the presubiculum (area 27 of Brodmann, HD 1 of Economo). This in turn continues as the subiculum (HE 1α and HE 1β of Economo) to the level of the lower border of the lateral ventricle, where the subiculum becomes continuous with the layer of ammonic pyramids.

There is quite severe degeneration in the fibres among the small densely packed nerve cells in the presubiculum. Degenerating fibres can be seen to enter this cortex from the more medially placed white matter, which is continuous behind the splenium of the corpus callosum with the cingulum bundle. The supracallosal striae immediately ventral to the corpus callosum appear normal.

Fibres within and emanating from the additional lesion in area 6 are swollen but not obviously degenerated. Moreover, that part of the internal capsule occupied by the descending projection from area 6 is normal. Although these observations suggest that the degeneration seen in the fibres from the cingulum springs from the lesion in the anterior cingulate gyrus, a second ablation of area 24 was performed to clarify the degeneration picture (case 2).

Case 2 (M 84). In order to limit the extent of the lesion more accurately than in case 1, the anterior cingulate cortex was ablated with a fine scalpel. The lesion in this brain is more anteriorly placed and much smaller than in the first experiment. Only a small part of area 24 and a small part of area 25 are involved. At its anterior end the lesion is restricted to the superficial cortical layers, and becomes narrow and slit-like at its posterior end (Text-fig. 2).

Terminal degeneration is present in the fibres in the same band of presubicular cells as in case 1, but is more obvious in the middle and towards the lower end of this strip of closely grouped cells. The swollen boutons are closely clustered round the nerve cells. A few degenerating fibres are present in the white matter lateral to the presubiculum and probably spring from the cingulum, but the supracallosal striae, which intervene between the presubiculum and the ventral aspect of the corpus callosum, appear normal.



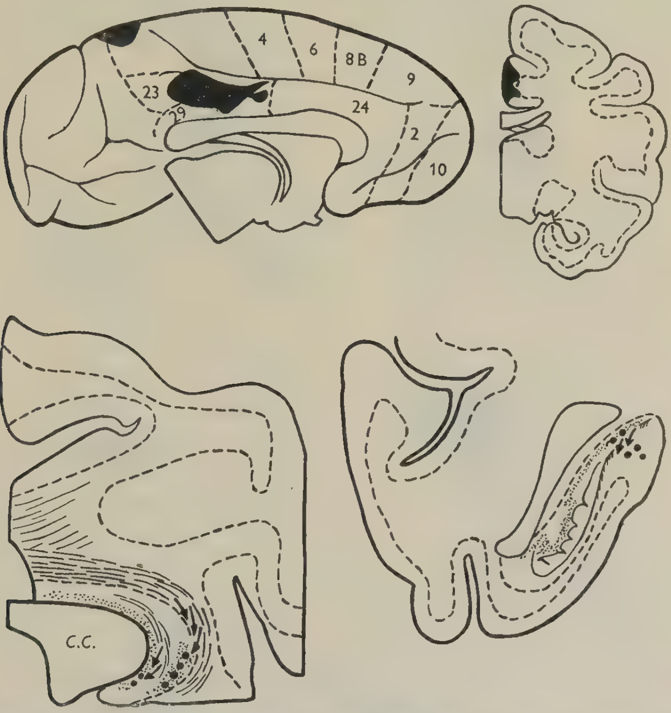
Text-fig. 2. Anterior cingulate ablation, case 2 (M 84). Terminal degeneration is present in the posterior cingulate cortex and adjacent to the presubicular cells ventral to the corpus callosum. Degenerating fibres can be seen entering this region from the overlying white matter. *C.C.* = corpus callosum.

Dorsal to the corpus callosum there is degeneration in the cortex of the cingulate gyrus, more severe ventrally, particularly in the cortex at the lateral end of the callosal sulcus (areas LE 1 and LE 2 of the retrosplenial cortex). Fine fibres undergoing degeneration are visible in the superficial layers in these areas. The indusium griseum and the supra-callosal striae appear normal.

B. *Ablation of the posterior cingulate area*

The survival period following ablation in this case (M 89) was 5 days. The lesion is confined to the cortex of the posterior cingulate gyrus and extends posteriorly to within 5 mm. of the splenium of the corpus callosum. At its posterior end it is restricted to the superficial cortical layers of the upper part of the cingulate gyrus (Text-fig. 3). There is a small lesion in the peristriate cortex at the supero-medial margin of the hemisphere resulting from a small cerebral hernia.

In order to obtain as complete a picture as possible of the fibres from the ablated area running in the cingulum bundle, sections were cut sagittally in the posterior cingulate region from a thin block of cortex which included only the cingulate cortex and a little of the subjacent white matter. These sections were examined for degeneration in the suprasplenial and retrosplenial portions of the cingulum.



Text-fig. 3. Posterior cingulate ablation (M 89). A few degenerating fibres are present in the cingulum. Degenerating terminals are scantily distributed in the retrosplenial cortex and in the presubiculum.

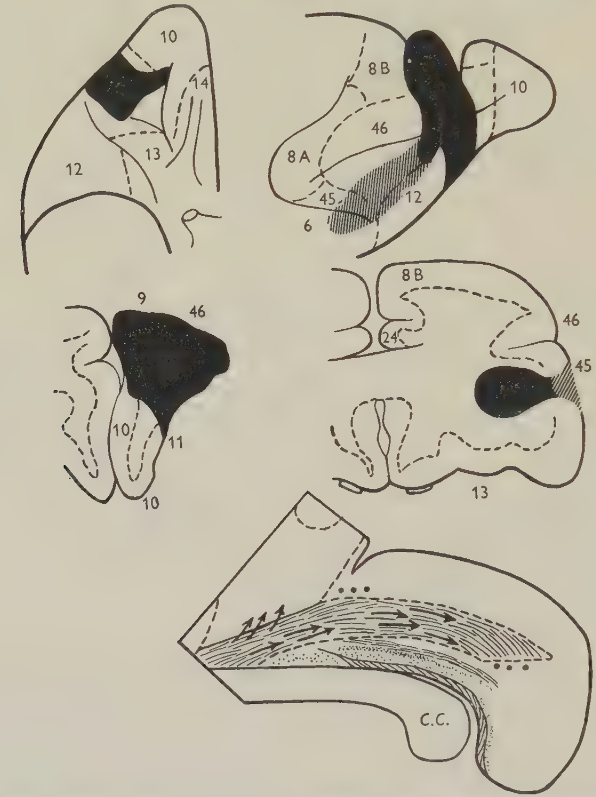
Transverse sections were then cut at the level of the splenium of the corpus callosum to assist in the examination of the presubiculum and adjacent regions lying ventral to the corpus callosum.

In the sagittal sections scattered degenerating fibres are present in the more ventral fibres of the cingulum. The dorsal part of the cingulum is normal. Fine fibres undergoing degeneration can be seen to leave the ventral aspect of the cingulum. These fibres end in the retrosplenial region near cells which in Nissl sections appear continuous ventrally with the presubiculum. There is some degeneration in the fine fibres of the supracallosal striae, and swollen and fragmented terminals are visible around the cells of the indusium griseum placed between the corpus callosum and the supracallosal striae.

In the transverse sections (Text-fig. 3) scattered degenerating fibres are present in the cingulum bundle ventral to the corpus callosum and immediately deep to the retrosplenial cortex. Terminal degeneration can be traced into the presubicular strip adjacent to this part of the cingulum, and is also present in the deeper layers of the retrosplenial cortex. The over-all picture is one of surprisingly little degeneration after a lesion of considerable size in cortex which has been described as making a significant contribution to the cingulum bundle (Cajal, 1911).

C. *Ablation of prefrontal areas*

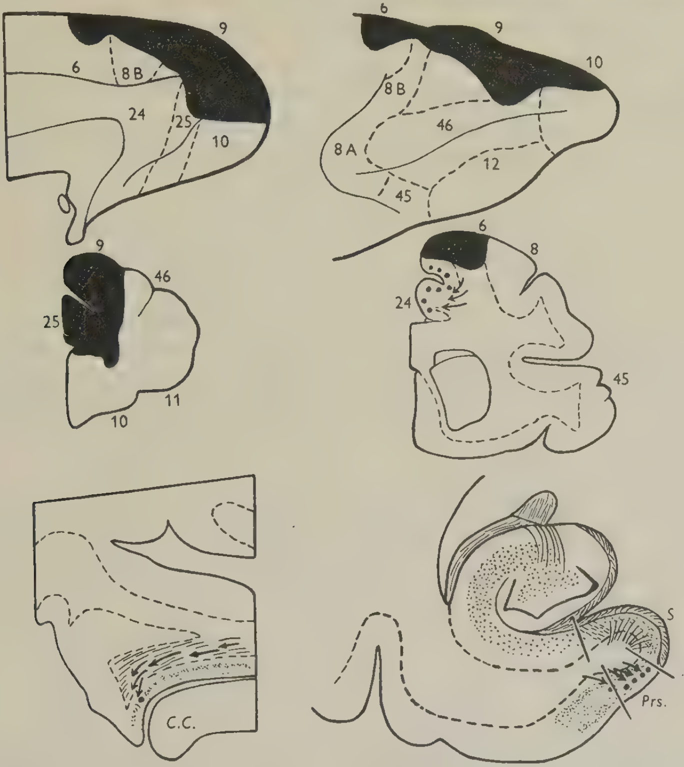
Case 1 (M 82). This animal was killed 6 days after operation. All granular areas of the frontal lobe have been damaged, the tip of the frontal pole being almost severed from the rest of the brain (Text-fig. 4). The lesion is considerably anterior



Text-fig. 4. Prefrontal ablation, case 1 (M 82). The hatched area indicates the subcortical extension of the lesion. Numerous degenerating fibres are present in the cingulum, some of which leave the bundle to enter the cortex of area 23. Some terminal degeneration is present in the retrosplenial cortex.

to the frontal eye field, and microscopically the agranular cortex of the upper part of area 6 and of the posterior orbital surface (area 13) has been spared. The lesion extends beneath the surface, however, into the inferior part of area 6, a type of cortex which von Bonin & Bailey (1947) have identified as FCBm (Brodmann's area 44), a dysgranular type of cortex.

In sagittal sections which include the posterior cingulate and retrosplenial regions, considerable numbers of degenerating fibres are visible in the cingulum, scattered diffusely from the dorsal to the ventral aspects of the bundle. Dorsally, degenerating fibres leave the cingulum to enter the cortex of area 23. Degenerating terminals can be seen around the cells of this cortex adjacent to the cingulum. A few fine fibres (finer than in the cingulum) are degenerating in the white matter dorsal to the cingulum. The degenerating cingulate fibres can be traced posteriorly to the retrosplenial portion of the cingulum. Occasional degenerating terminals are present in the upper part of the retrosplenial cortex. The supracallosal striae appear normal, and no terminal degeneration can be found in the indusium griseum, although in most specimens this has not been very heavily impregnated by the silver stain.



Text-fig. 5. Prefrontal ablation, case 2 (M 92). A more restricted lesion than in the first case. Degenerating fibres in the cingulum can be traced to the retrosplenial region (lower left) and terminal degeneration is considerable in the presubiculum (lower right). Fibres can also be traced from the lesion into the anterior cingulate area (middle right). *C.C.* = corpus callosum. *Prs.* = presubiculum. *S.* = subiculum.

Case 2 (M 92). This animal was killed 7 days after operation. The lesion is much less extensive than in the previous case (Text-fig. 5). It is situated mainly in areas 9 and 10, both on the medial and lateral aspects of the hemisphere. Medially the lesion extends below into area 25 and involves very slightly the anterior border of area 24. On the lateral surface the inferior margin of the lesion extends slightly into the anterior part of area 46. An additional small lesion was placed in the medial part of area 6, involving the cortex adjacent to the dorsomedial border of the hemisphere on both the lateral and medial surfaces. The tip of the frontal pole is intact.

As in the previous experiment, sagittal sections were first cut from a thin block of tissue in the region of the callosal splenium, and transverse sections subsequently obtained from the posterior end of the hippocampal formation.

In the sagittal sections there are considerable numbers of degenerating fibres in the posterior part of the cingulum (Text-fig. 5). They are of medium size and lie mainly in the ventral part of the cingulum. In medial sections they are more numerous above the splenium of the corpus callosum than posterior to it, indicating that they swing laterally as they round the splenium. Fine degenerating fibres can be seen leaving the retrosplenial part of the cingulum to enter the deeper layers of the indusium griseum. No degenerating fibres were found in the retrosplenial cortex, nor in the white matter dorsal to the cingulum.

Transverse sections of the posterior end of the hippocampal region show numerous degenerating terminal boutons in the third layer of the presubiculum. The cells in this layer lie among both horizontally and radially directed fibres. Fine fibres entering the deeper layers of the lateral part of the presubiculum and also the adjacent entorhinal area from the overlying white matter are fragmented. The degeneration is slightly more severe at the lateral end of the presubiculum than the medial, but ceases fairly abruptly at the lateral border of the presubiculum, although occasional degenerating terminals are present in the adjacent entorhinal cortex.

Transverse sections in the anterior cingulate region at the level of the lesion in area 6 show degenerating fibres running downward from the lesion in the white matter immediately deep to the cortex of the medial part of area 6 (Text-fig. 5). These fibres can be traced around the deep end of the cingulate sulcus; they terminate radially in the outer layers of the cortex on the inferior lip of the cingulate sulcus. Some of the fibres appear also to enter the cingulum, but, as the cingulum has been sectioned transversely, the presence of degenerating fibres within it could not be determined with certainty. Degenerating terminals are also present in the outer layers of the cortex on the superior lip of the cingulate sulcus. The cortex on the free surface of the cingulate gyrus (area 24) shows degenerating fine fibres in its third layer. The indusium griseum appears normal.

DISCUSSION

The origin of the cingulum bundle has been extensively studied in normal material. The intimate relationship between the cingulum and the cingulate cortex was first postulated by Beevor (1891). He suggested that numerous short tracts in the cingulum connect adjacent portions of the cingulate cortex, and that it is not possible, even after complete section of the cingulum at any one level, to produce degeneration in all its fibres.

Our monkey material confirms this opinion in that lesions in medial prefrontal, anterior cingulate and posterior cingulate cortex each produced degenerating fibres in varying numbers in the cingulum at posterior levels, but the aggregate of such degenerating fibres constitutes only a small proportion of the total fibres of the cingulum at the posterior cingulate level. Beevor envisaged a portion of the cingulum arising anterior to the genu of the corpus callosum, and considered this to be connected with the medial root of the olfactory tract. Such a connexion, however, appears to be negatived by recent experimental studies (Le Gros Clark & Meyer, 1947; Meyer & Allison, 1949). Our study shows that many of the fibres of the cingulum arising anterior to the genu of the corpus callosum do so from the medial prefrontal cortex, and that a greater number of fibres from this cortex reach the splenium of the corpus callosum than from anterior or posterior cingulate cortex.

Cajal's (1911) preparations of the cingulum in small mammals showed that, in addition to the short tracts described by Beevor, there is a very long tract running its whole, or almost its whole, length. Nevertheless, the fibres of the cingulum in these animals are stated to arise mainly in the cells of the cingulate cortex. Cajal considered that the white cord situated above the corpus callosum is not entirely the homologue of the human cingulum. In the mouse and rabbit it has distinct external and internal fasciculi: the internal fasciculus has thick fibres arising in the cells of the inter-hemispheric cortex and is probably equivalent to the human cingulum, while the external part is larger than the internal and forms an association path between the anterior and posterior surfaces of the hemisphere. Its thin fibres have no connexions posteriorly with the hippocampal formation* and it probably corresponds to the superior longitudinal fasciculus of Burdach in man.

Cajal described three groups of fibres in these animals in the internal fasciculus or cingulum proper, each group arising from cells of the inter-hemispheric cortex. There are posterior fibres running towards the splenium of the corpus callosum and ending in the retrosplenial cortex. Secondly, anterior fibres, much more numerous than the posterior, run forward around the genu of the corpus callosum. The bulk of the cingulum, however, is stated to be formed in these animals by bifurcating axons which send branches of equal size both anteriorly and posteriorly. He found that the majority of the fibres running posteriorly end in the subiculum and hippocampus. Turning round the splenium of the corpus callosum, they run to the subiculum, enter its first layer and mingle with fibres of the great crossed temporo-ammonic tract. They end among the cells of the hippocampus and fascia dentata. He considered that the supracallosal striae (nerves of Lancisi), lying ventral to the cingulum, arise in the rudimentary cortex of the indusium griseum and, like the fibres of the cingulum, are formed of bifurcating fibres which divide into anterior and posterior branches..

The cortex of the cingulate area has been studied architectonically in normal and experimental material. Le Gros Clark & Boggon (1933) demonstrated in Marchi preparations and by the method of retrograde cell degeneration that this cortex in the cat and rat receives fibres from the anterior nuclei of the thalamus. A careful comparative study by Rose & Woolsey (1948) in the rabbit and cat indicates that the projections from the anterior thalamic nuclei provide a basis for subdividing the cingulate cortex; the anteromedial nucleus projecting to the anterior cingulate area, the antero-ventral nucleus to the posterior cingulate area, and the antero-dorsal nucleus to the retrosplenial cortex.

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* The term 'hippocampal formation' is taken here to include the subiculum and presubiculum as well as the hippocampus proper.

Although Rose & Woolsey's retrograde degeneration methods did not allow a direct examination of fibre pathways, they have been able, by a careful comparison of the extent of lesions in different animals, to suggest the probable course of fibres in the rabbit and cat from the thalamus to the medial wall of the hemisphere. They believe that fibres from the anterior thalamic nuclei enter the internal capsule and run forward for some distance before turning dorsally and medially towards the medial wall, and that possibly they then enter the cingulum.

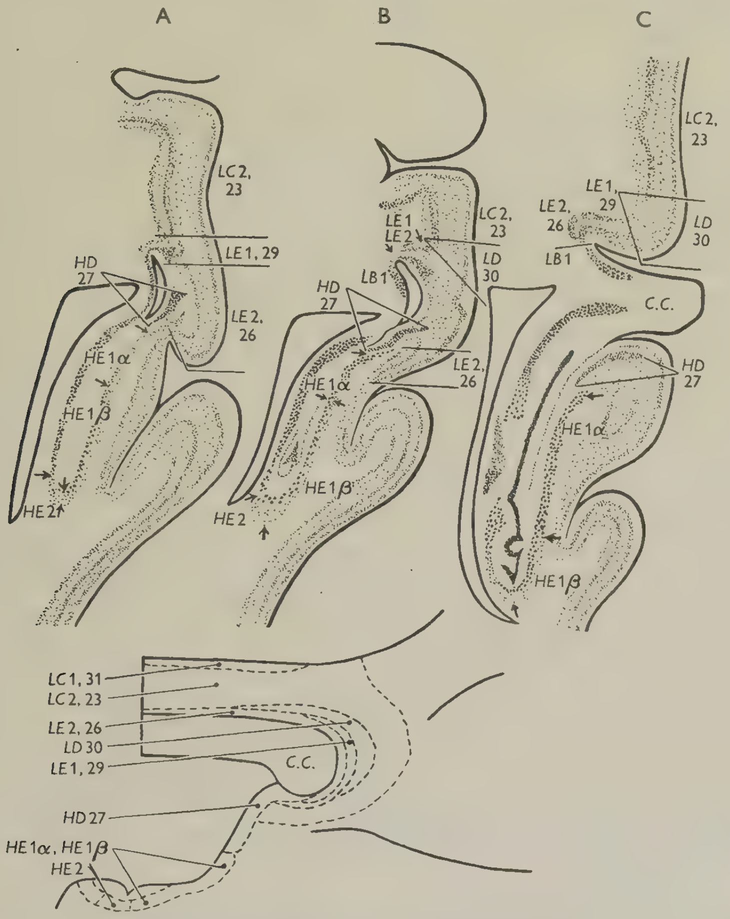
Just how far forward such fibres from the anterior thalamic nuclei loop in the primate brain before running to the cingulate cortex is not known, and the possibility must be considered that even our restricted prefrontal ablation (M 92) may have directly involved such fibres. However, transverse sections through the anterior part of the frontal lobe show that the white matter is involved only near the tip of the lobe, and the damage to white matter decreases very rapidly at more posterior levels, that underlying the pre-genual portion of area 24 and most of area 25 being uninjured. The lesion involves the upper part of area 25 and the subjacent white matter is slightly damaged (Text-fig. 5). It is therefore unlikely that the degeneration observed in the cingulum in this case at posterior levels results from direct damage to the thalamic projection fibres.

Rose & Woolsey found that in the rabbit and cat the cortex of the anterior limbic region is essentially agranular, the posterior limbic region has an obvious granular layer, while the retrosplenial cortex has a thinner sixth layer than the posterior cingulate cortex and granular cells spread into the supragranular layers. M. Rose (1928) examined the retrosplenial cortex in the chimpanzee and divided it into a granular area (RSg) and an agranular retrosplenial area (RSag). He further subdivided the granular retrosplenial area into medial, intermediate and lateral portions ($RSg\alpha$, $RSg\beta$, $RSg\gamma$), but there can be little justification for such additional subdivisions in the absence of evidence of differing functional connexions.

The same criticism may be levelled at our own study of the cytoarchitecture of the cortex in this region in normal Nissl material (Text-fig. 6). Examination of this area in the macaque shows that the differentiation approaches more closely the intricate arrangement found by Economo (1929) in the human than the simple pattern depicted for *Cercopithecus* by Brodmann (1909). Lorente de Nó (1933) has pointed out the futility of cortical studies based solely on cytoarchitecture without regard for fibre connexions; the arrangement shown in Text-fig. 6 is presented only to assist in identification of the cell groups in which degeneration has been found, and to point out the continuity of the presubicular cell column in the region below the splenium of the corpus callosum with closely packed cells of very similar appearance extending some distance into the retrosplenial area.

The earliest experimental study of the origin of the fibres of the cingulum is that of Probst (1903) in the cat. After dividing the bundle in the supracallosal part of its course, he found Marchi granules extending both anteriorly and posteriorly from the level of section, the degenerating posterior fibres reaching as far as the hippocampus. No degeneration was found, however, if the lesion involved the cortex of the hippocampal gyrus, the ammonshorn, the olfactory bulb or motor cortex. He therefore concluded that the fibres of the cingulum arise from interhemispheric cortex.

More recently, Gardner & Fox (1948) interrupted the cingulum of the monkey in area 24 with a stereotaxic instrument and studied the ensuing degeneration by the Marchi method. Only a few degenerating fibres running posteriorly enter areas 18 and 19, but most curve round the splenium to enter the subiculum. They found Marchi granules close to the hippocampus, in its medial postero-superior part, and their reduced silver preparations strongly suggest the possibility of direct connexions with the hippocampus. They point out that the cortex caudal and medial to the hippocampus constitutes the *écorce temporale supérieure ou postérieure* of Cajal, or entorhinal area of Brodmann, which is the main source of afferent fibres to the



Text-fig. 6. Cyto-architectural arrangement of the cortex in the retrosplenial region of *Macaca mulatta* as seen in coronal sections of normal Nissl material. Figures A, B and C are at successively more anterior levels. A reconstruction of the main areas as seen in these sections is shown below. *HD*, 27 = presubiculum. *HE* 1α and *HE* 1β = subiculum. *HE* 2 = area pyramidalis ammonica. *LB* 1 = area indusii. *LC* 1, 31 = area cingularis posterior dorsalis. *LC* 2, 23 = area cingularis posterior ventralis. *LD*, 30 = agranular retrosplenial region. *LE* 1, 29 = superior granular retrosplenial area. *LE* 2, 26 = inferior granular retrosplenial area.

hippocampus. They did not determine the origin of the fibres of the cingulum. Our preparations show, however, that fibres of the cingulum arising in prefrontal, anterior and posterior cingulate areas terminate mainly in the presubiculum of the hippocampal formation, and that these fibres enter the adjacent entorhinal area only to a limited degree. A number of degenerating fibres could be seen entering the medial part of the entorhinal cortex after a prefrontal ablation (M 92), but after anterior and posterior cingulate ablations no such terminations were detected.

Glees *et al.* (1950) traced degenerating fibres in the cingulum as far as the retrosplenial area after lesions in area 24 which either involved the cingulum directly (MCG 1) or were accompanied by lesions in adjacent frontal cortex (area 9 in MCG 1, the medial part of area 6 in MCG 5). Our first ablation of area 24 (M 81) was also accompanied by direct damage to the cingulum, and in the second experiment (M 84), where a more restricted lesion did not involve the cingulum, there were significantly fewer degenerating fibres observed in the retrosplenial region.

Physiological neuronography has yielded information on the functional organization of the medial aspect of the primate cortex. Bailey *et al.* (1944) strychninized this cortex in the macaque and chimpanzee. They found a 'cingular belt' along both dorsal and ventral lips of the cingulate sulcus which receives fibres from the suppressor strips (8*s*, 4*s*, 2*s* and 19*s*) and expands anteriorly to include most of area 32 as defined by Brodmann. They strychninized area 10 in the chimpanzee, but did not detect any firing from this area into the posterior cingulate or retrosplenial regions. Dunsmore & Lennox (1950) also observed projections to area 31 in the 'cingular belt' of Bailey after strychninization of area 24 in the macaque, but no spikes were reported from areas 23 or 29. Pribram, Lennox & Dunsmore (1950) evoked strychnine spikes in the hippocampus by stimulation of the retrosplenial area and posterior end of the entorhinal area, but stimulation of the posterior cingulate area produced only local firing.

The failure of the neuronographic method to show the connexions from granular frontal and anterior cingulate areas to retrosplenial and hippocampal regions as seen in our silver preparations may be accounted for in part by the difficulty of exposing the latter regions to the recording electrodes. Consideration must also be given to the recent findings of Frankenhaueser (1951) that regions of the brain such as the olfactory bulb and vagal nuclei are unresponsive to strychnine. Negative findings from neuronography do not therefore preclude the existence of important anatomical connexions.

Evidence is growing from degeneration studies in human leucotomy material that considerable numbers of fibres run caudally in the cingulum from the frontal lobe. Meyer *et al.* (1947), relying on the gross evidence of gliosis studies, traced degeneration in the cingulum only 3 cm. behind the level of section. Beck (1950) has studied the gliosis in more extensive material, and traced it as far as the splenium, but could find no degeneration in the hippocampal region. A. Meyer (1950) found that in cases where the leucotomy involves complete isolation of the prefrontal cortex, evidence of degeneration is seen not infrequently in the cortex of the anterior cingulate region. Our findings in the monkey (M 92) confirm the presence of fibres from prefrontal cortex terminating in the anterior cingulate area (Text-fig. 5).

Stimulation and ablation studies in the medial prefrontal and cingulate areas have been carried out in animals and man (Smith, 1945; Ward, 1948*a*, *b*; Dunsmore & Lennox, 1950, in the monkey; Kremer, 1947; Clark, Chow, Gillaspy & Klotz, 1949, in the dog; Pool & Ransohoff, 1949, in man). Respiratory and vascular responses have been stated to follow stimulation of the anterior cingulate area; also suppression of evoked activity in somatic musculature (Kremer, 1947; Ward, 1948*a*, *b*; Dunsmore & Lennox, 1950) and suppression of strychnine spikes (Dunsmore & Lennox, 1950) have been reported, although Clark *et al.* have been unable to demonstrate suppression in unanaesthetized dogs after stimulation of the anterior cingulate region.

In addition to these autonomic effects, certain changes in behaviour have been reported following ablation of the medial prefrontal and anterior cingulate areas. In his original discussion of the anatomy of emotional control, Papez (1937) suggested that the hypothalamus, the anterior thalamic nuclei, the gyrus cinguli, the hippocampus and their interconnexions constitute a harmonious mechanism concerned in emotional control. Bard & Mountcastle (1948) have shown that cats subjected to complete ablation of the neocortex, but with the 'midline' cortex intact, become abnormally placid; subsequent removal of the cingulate cortex converts the placid state into an extremely irritable one and Ward (1948*a*, *b*) reported that ablation of area 24 in the monkey leads to a loss of fear of man, social indifference to fellow monkeys, and loss of mimetic activity, grooming and signs of affection. Glees *et al.* (1950) have noted a similar indifference to cage-mates after bilateral ablations of area 24, but they found that these changes are temporary, disappearing completely in an animal allowed to survive for 3 months after operation.

Fulton (1949) suggested that the common factor in many successful cases of frontal leucotomy might be a lesion in the cingulate area. However, Glees *et al.* found that ablation of area 24 in advanced psychotics produced little temporary or permanent behaviour change, although in obsessional psychoneurotics there appeared to be considerable improvement. There is no dramatic improvement such as is at times observed after the standard method of leucotomy. On the other hand, Freeman (1948) and Pool (1949) claim that clinical improvement after a topectomy involving mainly the medial parts of the frontal lobe is comparable with that after leucotomy. Freeman considers that the efficacy of the procedure of transorbital leucotomy lies in undercutting areas 9 and 10, thereby dividing the thalamo-frontal radiations to these areas. Poppen (1948) also is careful to ensure that all white matter is divided on the medial inferior quadrant to the lateral ventricle, the white digitations in the medial frontal gyri being interrupted.

There has been a strong tendency to interpret the beneficial effects of frontal leucotomy in terms of the interruption of thalamo-cortical pathways, particularly those from the dorsomedial thalamic nucleus. Yakovlev, Hamlin & Sweet (1950) point out that the degeneration of the anterior thalamic radiations following frontal lobotomy has been stressed to the exclusion of the even more obvious degeneration in their material in the far greater mass of efferent projections which connect the frontal lobes to all levels of the neuraxis. They consider that these efferent systems are equally important and must be taken into consideration in the construction of any anatomical scheme correlating the frontal lobotomies with the subsequent re-organization of behaviour. The existence of an important contribution to the hippocampal formation from 'granular frontal cortex through the cingulum bundle offers a possible explanation of the satisfactory results obtained by Freeman after transorbital leucotomy and the relatively indifferent results following topectomy of area 24. Simple removal of anterior cingulate cortex would not necessarily involve the underlying cingulum bundle. Those of its fibres arising in the granular frontal cortex of areas 9 and 10 would be cut, however, in the procedure of transorbital leucotomy.

The postulated role of the hippocampus itself in emotional mechanisms can hardly be said to be established with certainty as yet, even though the results of recent experimental studies appear to provide confirmatory evidence. For example, Bard & Mountcastle (1948) reported that removal of the hippocampus in the cat leads to increased pleasure reactions. Bucy & Kluver (1940) carried out extensive temporal lobectomies in the monkey which included the major part of the hippocampus, and noted profound changes in behaviour which they summarized in the term 'psychic blindness'—a lack of appreciation of possible dangers or pleasures in objects handled. The animals became extremely docile and failed to retaliate when abused by their fellows. They exhibited hypersexuality and extreme oral tendencies, any and every object examined being placed in the mouth. It remains uncertain, however, whether these behavioural changes in monkeys were due entirely (or even mainly) to the involvement of the hippocampus.

If further inquiry should establish an essential relationship between hippocampal functions and emotional behaviour, the fronto-hippocampal fibres of the cingulum which have been demonstrated by our experimental studies may be found to provide one of the efferent tracts from the frontal lobe involved in emotional control.

SUMMARY

1. The origin of certain fibres running in the cingulum to the hippocampal region has been examined in five monkeys (*Cercopithecus sabaeus*), by the use of a silver staining technique to show degenerating fibres and terminals.

2. After lesions in the anterior cingulate area degenerating fibres can be traced in the cingulum to the presubiculum of the hippocampal formation. Some of these fibres also terminate in the posterior cingulate area.

3. From the posterior cingulate area fibres can also be traced in the cingulum to the retrosplenial cortex and the presubiculum. The number of such fibres reaching the presubiculum appears to be less than from the anterior cingulate area.

4. Lesions in the granular prefrontal cortex mainly on the medial aspect of the hemisphere result in a greater number of degenerating fibres in the cingulum than after either anterior or posterior cingulate ablations. These fibres terminate in the presubiculum and the adjacent part of the entorhinal area.

5. The significance of direct prefronto-hippocampal connexions is discussed in view of the possible importance of such a pathway in mechanisms of emotional control.

We wish to express our thanks to Prof. W. E. Le Gros Clark for his continued help and encouragement. Mr L. Parke and Mr R. Laing have given valuable assistance in staining the histological material. We also wish to thank Prof. A. A. Abbie in the Department of Anatomy, University of Adelaide, for the provision of photographic facilities.

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EXPLANATION OF PLATE

A. A degenerating fibre in the retrosplenial portion of the cingulum after a prefrontal ablation (M 92). ×900.

B. Prefrontal ablation (M 82). Several degenerating fibres in the retrosplenial portion of the cingulum. A fragmented fibre with a club-like swelling is visible running downward and to the left from the centre of the field. ×800.

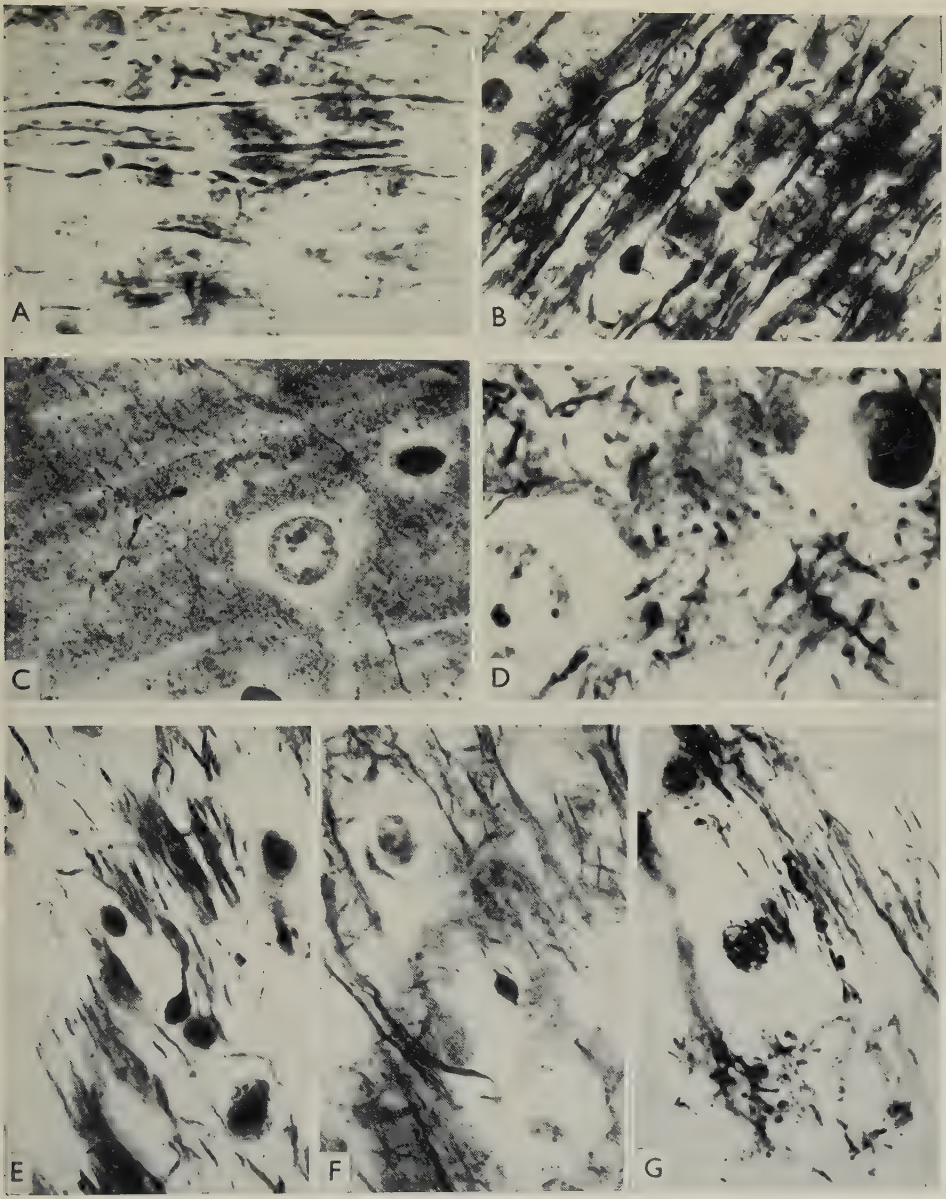
C. Degenerating terminal droplets in the vicinity of a nerve cell in the anterior cingulate area after prefrontal ablation (M 92). ×1000.

D. An aggregation of degenerating terminal droplets in the presubiculum are visible in the centre of the field, following prefrontal ablation (M 92). ×1500.

E. A degenerating fibre in the retrosplenial region after an anterior cingulate ablation (M 81), appearing as a very darkly staining pear-shaped body in the centre of the field. ×900.

F. Anterior cingulate ablation (M 81). A degenerating terminal in the vicinity of a cell in the presubiculum. ×1000.

G. Anterior cingulate ablation (M 81). A degenerating fibre in the presubiculum. ×1000.



W. R. ADEY AND M. MEYER—HIPPOCAMPAL AFFERENTS FROM CINGULATE AND PREFRONTAL AREAS

IN MEMORIAM

CECIL McLAREN WEST, M.C., B.A., M.B., B.A.O., B.Ch., Sc.D.

Professor of Anatomy, University College, Cardiff

The news of the sudden death of Professor C. M. West, on 23 July 1951, in distressing circumstances came as a great shock to his anatomical colleagues and the wide circle of his friends. Apparently in his usual good health, he had left Cardiff in the morning to attend a meeting in London, but had a heart attack in the train and died before he reached Paddington.

Professor West had been Head of the Department of Anatomy at Cardiff for 24 years and for the last 6 years Acting Editor of this Journal. His death, in his 59th year, is indeed a sad loss to British Anatomy.

Cecil McLaren West was born at Chorlton-cum-Hardy, Lancashire, on 8 February 1893, the son of Arthur Birt West. He was educated at St Peter's School, Weston-super-Mare, Blundell's School, Tiverton, and at the University of Dublin. From Trinity College he graduated B.A. in 1913, and obtained the degrees of M.B., B.A.O., and B.Ch. in 1915. He had served as Cadet Sergeant-Major in the University Officers' Training Corps and in August 1914, while still an undergraduate in Medicine, he was commissioned in the Special Reserve and placed in charge of the Medical Unit. On qualification he was gazetted Lieut. R.A.M.C., immediately joined for duty and served with distinction in Egypt, France and Italy until his demobilization in April 1919. After service with the 10th Field Ambulance at Ypres in the autumn and winter of 1916—memorable to the present writer for his unfailing cheerfulness and steadfast comradeship in arduous and dangerous duties—and a period of illness in hospital, West was transferred to Italy to serve with the British Heavy Artillery. For his devotion to duty in exposed positions on the Carso and during the retreat after Caporetto he was recommended for the Italian Bronze Medal for Valour; and for his conspicuous gallantry during the great Austrian attack of June 1918 he was given the immediate award of the Military Cross in the field.

After post-graduate study in London and a year as Senior House-Surgeon at the West Kent General Hospital, Maidstone, West returned to Dublin as Chief Demonstrator of Anatomy under the late Professor A. Francis Dixon at Trinity College. In 1922 he was elected to the office of University Anatomist, a post that confirmed his choice of Anatomy as a career.

In 1923 he published his first paper, on 'Arrested development of the thyroid gland' (*J. Anat.* **57**, 269)—an indication of his early and lasting interest in embryological problems. The summer of that year was spent with the late Dr George L. Streeter at the Carnegie Laboratory of Embryology at Baltimore where West carried out an important investigation on 'The development of the gums and their relationship to the deciduous teeth in the human fetus'. His paper with that title, published in the *Contributions to Embryology* (No. 79), 1925, **16**, 23, has been frequently quoted and is now recognized as the classical account of an interesting subject.

In 1925 also he published 'A case of congenital cystic kidney' (*J. Anat.* **59**, 401), and in 1927 'A case of well-marked hypospadias' (*Irish J. med. Sci.* Jan.). In recognition of his published work and in appreciation of his services to the School of Anatomy in Trinity College as a very successful and inspiring teacher, the University of Dublin conferred on him the degree of Sc.D. in 1926.

From 1922 to 1924 West had been Secretary of the Section of Anatomy and Physiology of the Royal Academy of Medicine in Ireland, and he was President of that Section, 1924–6. He had joined the Anatomical Society of Great Britain and Ireland in 1921 and acted as Secretary for Ireland for some years.

In 1927, at the age of 34, West was appointed to the Chair of Anatomy in the University College of South Wales and Monmouthshire at Cardiff. There he amply confirmed and enhanced the reputation he had acquired in Dublin as a fine, inspiring teacher and a careful, painstaking investigator of anatomical problems. The reorganization and re-equipment of his Department on modern lines and other duties took up much of his time, but he did not fail to continue his original work and to guide and stimulate others in the paths of research. The preparation of his own published papers was never undertaken hastily, and they are consequently notable for the clarity of his exposition and the sound literary quality of his style. That quality is seen at its best in his sympathetic 'In Memoriam Notices' of his predecessors at Cardiff, David Hepburn (*J. Anat.* 1931, **65**, 551) and A. Francis Dixon, his former chief at Trinity College (*J. Anat.* 1936, **70**, 583): his tribute to the latter might well now be applied to himself. 'He brought to Cardiff a stimulating enthusiasm and energy which was one of his most striking characteristics....His work was done quietly and unobtrusively, and it is only now, when he is gone, that the debt to him, both in Cardiff and Dublin, is fully appreciated.'

At Cardiff, West continued his embryological interests. Apart from his contribution (with Lambert Rogers) on 'The Foramen of Magendie" (*J. Anat.* 1931, **65**, 457)—in which they reviewed the history of that 'eponymous and controversial hole' and, by special dissection and surgical observation, confirmed the trenchant claim of Wood Jones for its existence—and his Royal College of Surgeons Lecture on 'The female breast and its development' (*Ann. Roy. Coll. Surg.* 1949, **4**, 317), his chief publications are concerned with the description of early human embryos at different stages of development. These papers include complete accounts of human embryos of eight somites (*Contr. Embryol.* (No. 119), 1930, **21**, 25), and of twenty-five somites (*J. Anat.* 1937, **71**, 169); but the contribution to early human development by which he will be best known is certainly his description (with J. H. Dible) of 'A human ovum at the previllous stage' (*J. Anat.* 1940, **74**, 139 (*Proc. Anat. Soc.*, June, 1939); 1941, **75**, 269). The preliminary and definitive accounts of this important specimen belong by coincidence to the same years as the corresponding accounts of the first contribution of Hertig and Rock to early human development (*Anat. Rec.* 1939, **73** (Suppl.), 26; *Contr. Embryol.* (No. 184), 1941, **29**, 127). Dible and West therefore share with those authors the credit of demonstrating the now well-known 'primary yolk-sac stage' of human development first suggested by Stieve's 'Werner' ovum (1931, 1936). West's last formal contribution to human embryology, on 'Two presomite human embryos' (one of which he described at the International Anatomical Congress at Oxford in 1950), was in the press at the time

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of his death, and was expected to appear in the October number of the *J. Obstet. Gynaec.* 1951.

Apart from his published papers West contributed from time to time to the *Proceedings* of the Anatomical Society. On one memorable occasion at Cambridge (*J. Anat.* 1935, **69**, 145) he described to the Society, in his quiet and effectively humorous manner, his dissection under difficulty of a stranded whale (*Pseudorca*) and his extraction of a foetus. His preparation of the foetus and membranes was demonstrated but does not appear to have been published in detail. On other occasions he described a 'Medieval achondroplasic skull' (*J. Anat.* 1938, **72**, 621) and 'Human remains from Wookey Hole' (*J. Anat.* 1949, **83**, 86). He made numerous other Reports on human bones from the caves at Wookey Hole and elsewhere, some of which await publication in various Archaeological Proceedings. He was much in demand also as a popular lecturer to local scientific societies and other bodies. He enjoyed the reputation of being very unwilling to refuse an invitation, and the list of his titles is witness to the breadth of his interests in anthropology, human evolution and the history of anatomy.

West made several contributions to text-books. For the 4th edition of Jellett and Madill's *Manual of Midwifery* (1929) he rewrote Part I (Obstetrical Anatomy—Maternal and Ovular); and he revised the Section on the 'Skin and the Sensory Organs' for the 7th, 8th and 9th editions of *Cunningham's Text Book of Anatomy*: his revision for the last edition was still in the press at the time of his death.

Professor West's work as Acting Editor of this *Journal* since 1945, with the faithful assistance of his devoted secretary and friend, Miss Nancy D. Wood, has been notable for his business-like methods and his assiduous attention to all the details that make for judicious selection of papers and successful editing. Numerous contributors have reason to be grateful to him for his helpful suggestions with regard to style, the advantages of succinct statement of data and clarity of discussion, not to mention the sometimes much-needed lesson in the duty of authors to check their references and to state them correctly. These are the marks of the competent editor; and Professor West's services to the *Journal of Anatomy* and the Anatomical Society in that capacity, obvious from the high standard that has been maintained, have been fully recognized and gratefully acknowledged by his colleagues on the Editorial Committee. Since the *Journal* resumed the publication of Reviews, it may have been noticed that the editor has not been wanting in another of his duties that is sometimes irksome, the writing of such at short notice when other pens have not been available. He had thus contributed some thirty reviews during his editorship; of the three published in this number one was left in draft MS., and they now appear as his last contributions to the *Journal* that he served so faithfully and well.

Cecil West was a very lovable man. The admiration and affection of his students, both in Dublin and Cardiff, were his in full measure; and those who were near to him were aware that they were an unfailing source of happiness to him. With his staff his relations, founded on mutual trust, became intimately cordial. He never spared himself in his busy life of administration, teaching and research; but he was always ready with kindly and considerate advice for those who were privileged to work with him. The happy and harmonious atmosphere of his Department was evident to all who knew it; and the gracious welcome by Professor and Mrs West to those who took part in the Summer Meeting of the Anatomical Society at Cardiff in 1946 made it a very memorable occasion.

The unaffected grief of his many friends at the untimely passing of Cecil West has been a spontaneous tribute to his personality and capacity for friendship. To quote his own tribute to Francis Dixon again; Cecil West 'was a man whom it was a privilege to know, and whose friendship was a priceless treasure. May his memory never fade, but live as a shining example to every one of us who loved him dearly'. To his wife and family above all their bereavement has come as a sudden tragedy. He married Lucy Mary, daughter of the late Charles Joly, F.R.S., Royal Astronomer of Ireland, and herself a medical graduate; they had two daughters and a son; and our respectful and heart-felt sympathy has been with them all in their time of sorrow.

J.C.B.

PROFESSOR ALEXANDER MEEK

The death of Alexander Meek on 2 November 1949 severed yet another link with the great marine biologists of the nineteenth century. He studied for a year at the Royal College of Science and then at Dundee under Prof. D'Arcy Thompson. After taking his degree in 1889 he worked for two years with Prof. W. C. M'Intosh at the Gatty Marine Laboratory, St Andrews. He spent three years as a County Council Lecturer in Agricultural Zoology in Aberdeenshire, and then he obtained the post of Lecturer in Veterinary Anatomy and Farm Hygiene at the University of Durham College of Science, Newcastle upon Tyne. Four years later, in 1898, he was appointed to a demonstratorship in the Department of Natural History under Prof. G. S. Brady, whom he succeeded in the Chair of Zoology in 1908. The College of Science later became better known as Armstrong College, which was, in 1937, merged in the present King's College.

The north-east coast of England had for long been a centre of marine research, and here Meek, trained under D'Arcy Thompson and M'Intosh, was in his element. G. S. Brady and his brother H. B. Brady were both enthusiastic workers who had contributed to the Reports of the *Challenger* Expedition. Also in the neighbourhood and still very active was Canon A. M. Norman, whose name is, perhaps, even more distinguished in British marine biology. Through these there were links with the great men of the past such as Joshua Alder, Albany Hancock and many others.

Meek was very largely responsible for the establishment of the present Dove Marine Laboratory, founded in 1908 upon the ashes of a previous wooden laboratory burned down in 1904. He played a great part in continuing the tradition of research in marine biology on the north-east coast and paid particular attention to the problems of the in-shore fisheries.

Although there is no doubt that marine biology came first in Meek's scientific affections he was, throughout his life, keenly interested in anatomy and especially in embryology. He joined the Anatomical Society in 1918, and, at intervals throughout his active life, he published papers on a number of anatomical subjects in various journals, including the *Journal of Anatomy* and *Anatomischer Anzeiger*.

A.W.H.

OBITUARY NOTES

We regret to announce the death in May, 1951, at the age of 80, of Professor James Battersby, F.R.C.S., F.R.F.P.S. For many years he held the position of Professor of Anatomy at St Mungo's College of Medicine, Glasgow. We offer our sympathy to his wife and three sons.

We have heard with great regret of the death of Professor Thomas Walmsley of Queen's University, Belfast. A notice In Memoriam will appear in a latter issue.

We also regret to announce the death of Dr A. Peacock of the London Hospital Medical College. A notice In Memoriam will appear in a latter issue.

REVIEWS

Applied Anatomy for Nurses. By E. J. Bocock and R. Wheeler Haines. (Pp. 320; 231 illustrations; $8\frac{1}{2}\times5\frac{1}{2}$ in.; 15*s.* net.) Edinburgh: E. & S. Livingstone Ltd. 1951.

Although this book is intended primarily for nurses, one of its authors is an experienced and competent anatomist. This fact and the many good features that the book contains should make it of interest to readers of this *Journal.*

As has been noted, the book has the merit of brevity and cheapness. It gives a clear and straightforward account of the anatomy of the body, chiefly on a regional basis, and contains many humorous touches, new to anatomical texts, which help to lighten the reading and to impress facts on the student's mind. Indeed, the book might well form an introductory text of general anatomy for the medical student as well as for nurses or physiotherapists.

For example 'The radius may lie side by side with the ulna and parallel to it as in drinking from a cupped palm, when the forearm is said to be supinated, or the radius can be turned over the ulna so that the palm faces in the opposite direction as in kissing the back of the hand. This is pronation.'

In injury to the median nerve with loss of sensation and of protective response 'the first warning the patient has that his cigarette has burned down may be through the senses of smell or sight'.

There is a novel description of the pleura and the pericardium that may appeal to the student: 'In man the umbilicus is more conspicuous than in animals, and seems to have been specially elaborated as an ornament to a hairless and otherwise uninteresting abdomen.' There is an interesting paragraph on the appendix, and others on the voiding of flatus without faeces, on hob-nail liver and Kupffer's cells, psoas abscess, pararenal abscess, the arrangement of the cruciate ligaments of the knee; perhaps we should not all agree that the skin of the scalp 'is designed to absorb blows and to bear the pressure of the head on a hard pillow' though it may have to suffer both these indignities. Again, it is doubtful whether it can truly be said of the air sinuses that 'some of them make the skull more rugged in appearance (the Wellington face)'. There is an interesting suggestion that the arrangement for moistening and warming the air on its passage through the nose 'works well in warm climates, where primitive man was probably evolved, but poorly in cold, wet ones, where so many people suffer from periodic catarrhal inflammation'.

'A man can spurn the world with his foot, swear at it with his vocal muscles, or spit at it with his salivary secretions, but these are his only means of expression.'

All the illustrations are original, which in itself is refreshing. Some are very good, some are entertaining, for example one of a man carrying a brain on his back like a bundle and holding it in place with his arm which represents the greater wing of the sphenoid; or the flying skirt of the skating girl representing the folds of the mesentery that Professor Cunningham used to liken to a 'goffered frill'.

As a friend said to me when we were looking at the book together, 'The facts of life are fully illustrated.'

The nurses who read this book may be congratulated on their good fortune.

C. M. WEST

La Torsion Tibiale. By Paul-Victor Dupuis. (Pp. xi+195; 30 illustrations; $9\frac{1}{2}\times6\frac{1}{4}$ in; paper, 920 fr.) Liége: Desoer; Paris: Masson et Cie. 1951.

After an historical introduction the author deals briefly with the anatomical investigation of torsion in the tibia. He shows that there is a gradual change from a medial to a lateral torsion when followed from prenatal to postnatal life. There is a rapid increase in lateral torsion during the first year of life, then a stationary period during the second and third years, and the final condition is reached by the end of the fourth year.

The major portion of the work deals with the association of torsion with club-foot. There are some interesting points, too, with regard to the relation between torsion of the tibia and walking, and movement at knee and hip.

Finally, various methods of treatment, with case histories, are described.

The book is one that should certainly be consulted by those working on movements at the ankle and other joints of the lower limbs.

C. M. WEST

Traité d'Anatomie Humaine. By G. PATURET. Vol I (pp. 994, 588 figs., 5,200 fr., linen bound); Vol. II (pp. 1124, 741 figs., 6,000 fr., linen bound). Paris: Masson et Cie. 1951.

These are the first two of a series of six volumes that are to constitute a text-book of anatomy in which, as he states in his preface, Professor Paturet attempts to give not a new description of organs—'Car l'Anatomie ne varie pas'—but to present and interpret them in a new fashion. As he is a hospital surgeon as well as Professor of Anatomy at Clermont-Ferrand, the author sets out to stress the importance of the clinical application of anatomy, the study of the living body and the linking of anatomy and physiology. Nevertheless, his descriptive matter is extremely detailed, the whole accounting for the immense size of these two volumes.

The first deals with the bones (including the skull), joints and muscles, excluding the limbs which are dealt with completely in the second. Some idea of the thoroughness with which the upper limb is described may be gained from the fact that its account covers 516 pp. and is illustrated with 359 figs. Particular emphasis is laid on some structures, for example 20 pages are devoted to the shoulder joint, 30 to the diaphragm. The intervertebral disc is justifiably described in great detail over some 10 pages with 7 excellent illustrations, and special emphasis is accorded to fascial spaces.

In the classification of joints it is unusual to find included what the author calls 'Syssarcoses (de σύν, avec, et de σάρξ, chair, articulation chair contre chair) des espaces de glissement et non des articulations vraies'.

These volumes are amply illustrated with line drawings, mostly original and many schematic, with a generous allowance of colour; but the lack of radiographic illustration is surprising in view of the author's clinical outlook. Histology is not included.

The terminology employed is basically French, but B.N.A. and J.N.A. terms are sometimes inserted 'to facilitate the task of the foreign reader'. Apart from this, three or even four different names may be used for the same structure. For example, the serratus anterior appears as 'le grand dentelé ou serratus magnus ou grand scapulo-thoracique'. Eponymous nomenclature is rampant: eponyms, most of them completely unfamiliar to British readers, are freely scattered throughout the text and there are hundreds in the first volume alone. What a task for the student! What a prospect for international nomenclature! Yet the author is at pains to explain many of the ordinary French terms etymologically, though sometimes in a rather quaint fashion: for example, the scapula is 'L'omoplate (de ὦμος, épaule, et πλάτος, plat, c'est l'os "du plat de l'épaule")'. Biographical footnotes accompany many of the eponyms and there are many references to literature, also in footnotes.

In view of the size ($10\frac{1}{4} \times 8$ in.) and weight ($6\frac{1}{2}$ lb.; 7 lb.) of these two volumes, and the prospect of four more to follow, it is indeed surprising to find that the author states that he has written this text-book—the fruit of one man's labour for more than 20 years—particularly for students of medicine, though it is also addressed to specialists in general. It certainly bids fair to have many excellent features; but it is more than doubtful if students can have the time to give six such volumes the careful study they obviously require and deserve, even to read them through, not to mention the money to buy them. This text-book therefore cannot be recommended as suitable for students in this country; but it is certainly a work that should be available on the shelves of any well-stocked anatomical library. The index appears to be very good, though it may be a little difficult to use on account of the unfamiliar terminology.

C. M. WEST
J. C. BRASH

Über die Röntgenologischen Darstellungsmöglichkeiten des Weiblichen Genitalapparates mit Hilfe von Jodöl und Jodsol. By Dozent Dr. Med. Habil. J. Erbslöh. Archiv und Atlas der Normalen und Pathologischen Anatomie in Typischen Röntgenbildern, 1951. Stuttgart: Georg Thieme-Verlag.

This publication is essentially an account of techniques employed for the radiological demonstration of the female genital tract. The author makes an exhaustive examination of the viscosity and surface tension of the various contrast media and also their ability to moisten the tissues of the uterus and Fallopian tubes or amniotic sac. He also considers their possible effects on the patient. He concludes that iodized oils transformed into iodized colloidal solutions are the most suitable. While this publication is mainly of interest to the professional radiologist, it contains information of value to those interested in demonstrating the anatomy of the female genital tract in the living subject.

GEORGE SIMON

THE ANASTOMOSES BETWEEN INTERNAL AND EXTERNAL CAROTID CIRCULATIONS IN THE DOG

By P. A. JEWELL*

Department of Pharmacology, University of Cambridge

The present observations were undertaken as part of an investigation to localize the site of osmoreceptors in the dog. Verney (1947) has shown that such sensory elements exist somewhere within the field of distribution of the internal carotid artery. This conclusion is based upon experiments in which the output of anti-diuretic hormone was measured following the infusion of solutions, hypertonic to the blood, into carotid loops in dogs. It was found that such responses were temporarily abolished by ligation of the internal carotid artery near its origin.

It seemed desirable to know by what arterial pathways blood of common carotid origin might reach the brain, and, in particular, after ligation of the internal carotid artery near the carotid bifurcation, what alternative anastomotic channels might still carry carotid blood to the circle of Willis. Accordingly, a number of dissections of injected heads have been made in order to demonstrate the carotid vascular pattern.

The picture of the carotid circulation in the dog, that has evolved from these studies, is one that finds no adequate description in the literature. The standard anatomies of the dog (Ellenberger & Baum, 1891; Bradley, 1948; Sisson & Grossman, 1940; Miller, 1948) mention only the more striking features, and even the excellent text of Ellenberger & Baum is rendered inadequate by the fact that these authors followed too closely a supposed homology with human vessels, and thus left unemphasized some of the more important features of the dog's system. None of these works gives any indication of the functional significance of the numerous anastomoses which exist between intracranial and extracranial vessels, and there is a singular lack of illustration of their morphology.

The classical work of Julius Tandler (1899) gives a valuable description of the carotid circulation in the dog, although it is, unfortunately, an incomplete one. In particular, the nomenclature which he proposes, based on comparative studies of the head arteries in a number of mammalian species, is both accurate and acceptable where that of Ellenberger & Baum is not. Recently Davis & Story (1943) have published an authoritative account of the carotid circulation in the cat, and have directed attention to the large and functionally important anastomoses between intracranial and extracranial vessels which have been developed in this species to compensate for the absence of a patent internal carotid artery. Their work has provided a stimulating comparison for the present observations on the dog, and the close parallel that has been found between the patterns of the vessels in the two species emphasizes the necessity to reconsider the role of these anastomoses in the dog.

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MATERIAL AND METHODS

The description is based upon the dissection of heads of eighteen specimens. Many of the features described, particularly the form of the anastomotic artery, have been confirmed in a further series of animals which were principally used for histological purposes. Two injection masses were used: gelatin coloured with carmine or prussian blue, and an indian ink-plasma mass made by adding 10 % indian ink to human plasma reconstituted to three times normal strength. The method employed to inject the masses was that described by Verney (1947).

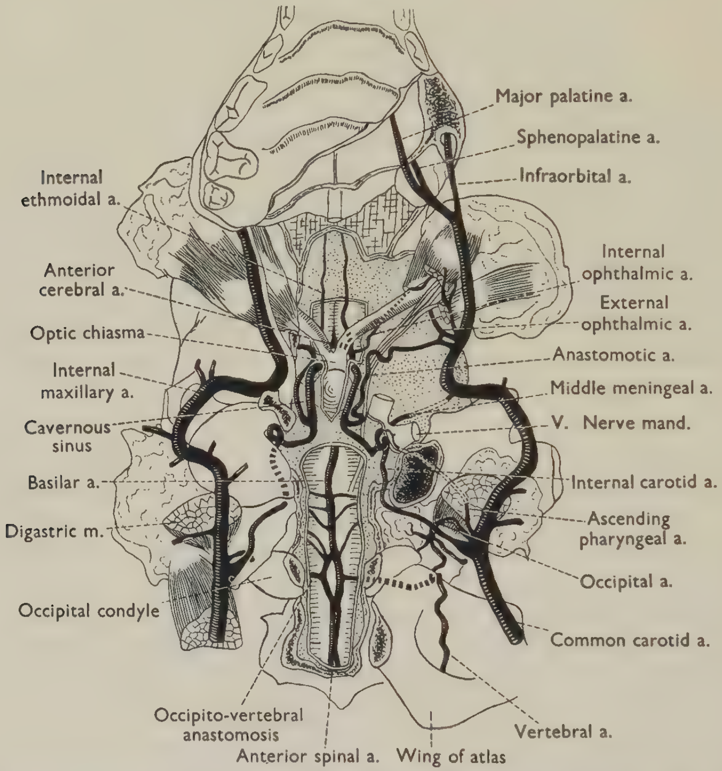


Fig. 1. Dissection of the dog's head to demonstrate the relationship between the arteries of supply to the brain. Ventral aspect; lower jaw and base of cranium removed. The external carotid artery is displaced laterally on the left side.

RESULTS

Five separate anastomoses linking extracranial and intracranial arteries have to be considered, although their relative importance as channels which may transport blood between the two systems is greatly different. The vessels concerned are depicted in Fig. 1, in which is shown their general relation to cranial and orbital structures, and to the vertebral arterial supply.

The anastomosis between occipital and vertebral arteries. The common carotid artery ascends to the level of the atlanto-occipital joint where it divides into internal and external carotid arteries. Immediately after its origin the external carotid artery gives rise to the occipital and ascending pharyngeal arteries. The origin of the occipital artery is very close to that of the internal carotid, being slightly lateral and ventral to the latter. For the first centimetre of their course the two vessels run nearly parallel, the occipital artery coming to occupy a position lateral to that of the internal carotid. As the artery approaches the occipital condyle it gives rise to an important branch which curls over the cranial border of the wing of the atlas to gain its dorsal surface, and there anastomoses with a parallel and terminal branch of the vertebral artery (Fig. 2). The vessel formed from the fusion of these two passes through the oblique foramen of the atlas, pierces the meninges, and runs across the ventral surface of the spinal cord to anastomose with the ventral spinal artery. The product of this union runs forward as the basilar artery to form the posterior limbs of the circle of Willis. The anastomosis was present in all specimens, and was of considerable size in some. It is not possible to say whether it normally carries a contribution of carotid blood to join the basilar flow, but it is certain that it will do so if additional demands are placed upon the basilar supply to the circle of Willis by operative interference with the internal carotid artery.

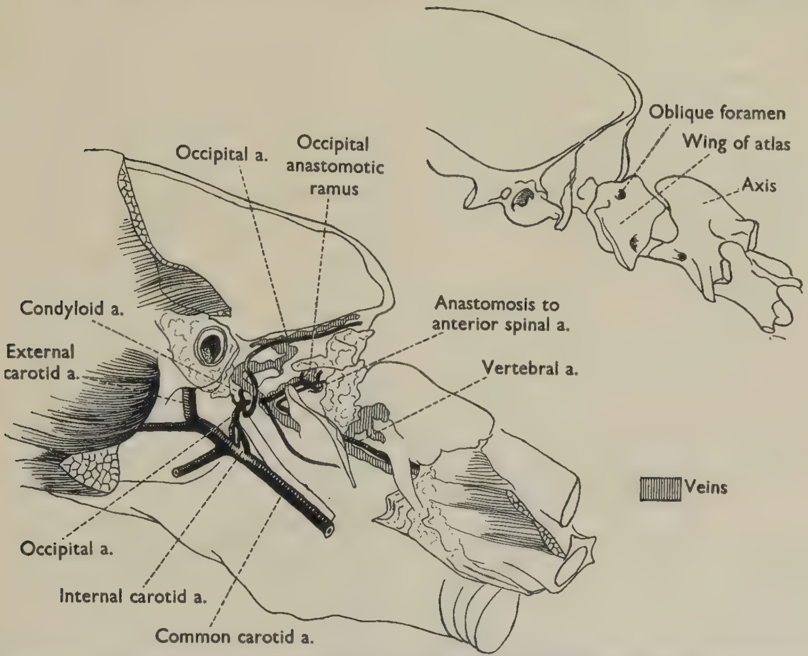


Fig. 2. Dissection of the occipital region of the dog's head to demonstrate the anastomosis between occipital and vertebral arteries. Left side, lateral aspect.

Neither Davis & Story (1943), nor Legait & Racadot (1949) make any mention of this anastomosis in the cat, and although it is present in other carnivores, existing comparative studies are inadequate to give any further clue to its functional significance.

The anastomosis between ascending pharyngeal and internal carotid arteries. Davis & Story (1943) drew attention to the unusual form assumed by the ascending pharyngeal artery in the cat. In this animal there is a rather remarkable development whereby the ascending pharyngeal artery performs the function of a supply route to the brain which the vestigial internal carotid artery has relinquished. The foramen lacerum medium is quite perforate and the ascending pharyngeal artery passes through it, from the roof of the pharynx, to reach the middle cranial fossa, where it contributes to the formation of the circle of Willis. These authors have shown, however, that the intracranial part of this vessel represents the distal end of the internal carotid artery which has assumed direct continuity with the ascending pharyngeal by the enlargement of an anastomosis through the foramen. I have confirmed their observation of the existence of this anastomosis in the dog, where a small terminal twig of the ascending pharyngeal artery pierces the thin fibrous covering of the foramen lacerum medium and joins the internal carotid artery. The internal carotid artery makes a tortuous loop within the depth of the temporal bone at this point, and the ascending pharyngeal anastomosis joins it at the apex of this loop (Figs. 1, 3 and 5*f*). It is safe to conclude that in the dog this anastomosis is of little or no functional significance. Indeed, according to Davis & Story, it is only in the domestic cat, amongst the carnivores, that it has any important role. In the bears and in the giant panda it is entirely absent. It is valuable to know of its existence, however, for it is one route by which the proximal segment of the internal carotid artery might be by-passed. No doubt it was this vessel that Bouckaert & Heymans (1935) were tying when they ligated the internal carotid and occipital arteries 'and the tissues between the origin of the internal and external carotids' in order to prevent blood reaching the brain from the region of the carotid bifurcation. When the proximal segment of the internal carotid alone is ligated in a survival experiment it is possible that this anastomosis would subsequently increase in size.

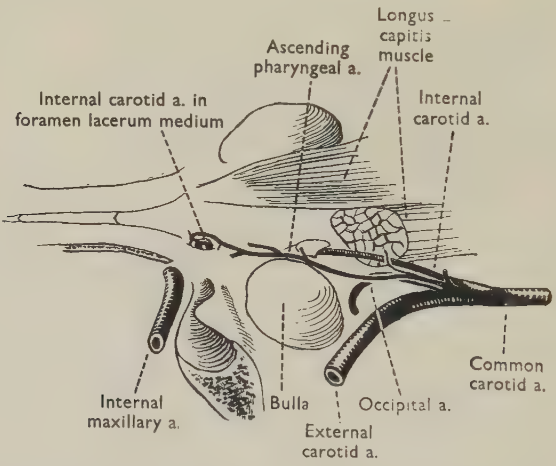


Fig. 3. The anastomosis between the ascending pharyngeal artery and the internal carotid artery through the foramen lacerum medium. Left side, ventro-lateral aspect.

The internal carotid artery itself, although considerably smaller than the external carotid, is a vessel of fair calibre in the dog. It runs forward from its origin at the carotid sinus to enter the carotid canal of the temporal bone. Upon emerging from the canal the artery enters the cavernous sinus and traverses its whole length before bending dorsally to pierce the meninges and form the circle of Willis. In the whole of this long and tortuous course the internal carotid artery gives no branches, except sometimes a small branch to the posterior lobe of the pituitary gland and nutrient twigs to the meninges. On the other hand, it receives two anastomoses—that from the ascending pharyngeal artery already described, and a large anastomosis originating from the internal maxillary artery.

Anastomoses between intracranial vessels and the internal maxillary artery. The branches of the external carotid artery to the lower jaw, the ear, and superficial temporal structures do not concern us here; but the continuation of the external carotid artery, distal to the superficial temporal artery, as the internal maxillary artery, is of singular importance for from it arise a series of anastomoses by which the extracranial and intracranial vessels are united.

The internal maxillary artery, coursing medially from behind the post-glenoid process to circumscribe the mandibular condyle, comes to occupy a position closely applied to the wall of the cranium. For a short part of its course it runs within the alisphenoid canal of the sphenoid bone and there gives rise to the middle meningeal artery, which runs into the cranial cavity in proximity to the emerging mandibular branch of the trigeminal nerve. The internal maxillary artery leaves the alisphenoid canal in association with the maxillary branch of the trigeminal nerve, and runs with it across the floor of the orbit. Almost immediately upon leaving the canal it gives rise to the orbital artery, which crosses the maxillary nerve dorsally and divides into the arteries of supply to orbital structures. It is concerning the names of these arteries, which form the orbital complex, that confusion has arisen in the literature, and it would seem best to discuss the nomenclature used here before proceeding further. The vessels are illustrated in Fig. 4.

The orbital artery was called by Ellenberger & Baum the 'ophthalmic' artery. As Tandler has shown, however, this artery is under no circumstances the homologue of the ophthalmic artery in man, which arises from the internal carotid artery; neither has it a true homologue in man. Further, it is found amongst the mammalia that the arterial supply to the orbit may come from many diverse sources—from the stapedial artery as in the marmots, from the occipital artery as in the armadillos, or from the internal maxillary artery as in the dog. The name ophthalmic is, therefore, best not used for these other arteries of supply to the orbit, but may be retained to designate that branch of the parent vessel which gives rise to the central artery of the retina. Tandler names the vessel arising from the internal maxillary artery to supply the orbit in the dog the *orbital artery*. This name is adopted here.

A short distance from its origin the orbital artery gives rise to a primary branch which turns immediately into the orbital fissure through which it runs to join the internal carotid artery in the cavernous sinus. This vessel is named by Ellenberger & Baum, after Bellarminov, the 'internal ophthalmic' artery. This is a complete misnomer, for not only does it suggest non-existent homologies, but it is quite misleading as to the function of the artery. Tandler names this vessel the *anastomotic artery*. It is contributory to, and not a branch of, the internal carotid artery.

The true homologue of the human ophthalmic artery was missed by Ellenberger & Baum. It is a small vessel that arises from the anterior cerebral artery and runs in company with the optic nerve through the optic foramen into the orbit. This vessel only was named by Tandler the ophthalmic artery. It anastomoses near the bulbus with a larger vessel that arises extracranially as a branch of the orbital artery. The anastomosis gives rise to the central artery of the retina. This branch of the orbital artery was given no specific name by Tandler, whilst Ellenberger & Baum appeared to regard it as a branch of the 'internal ophthalmic' artery. The vessel is best regarded as an extracranial parallel of the true ophthalmic artery, since from their union there arises the central artery of the retina. In conformity with recent authors (Sisson & Grossman, 1940; Miller, 1948) the names *internal ophthalmic artery* (for the vessel arising from the anterior cerebral) and *external ophthalmic artery* (for the vessel arising from the orbital artery) will be adopted here.

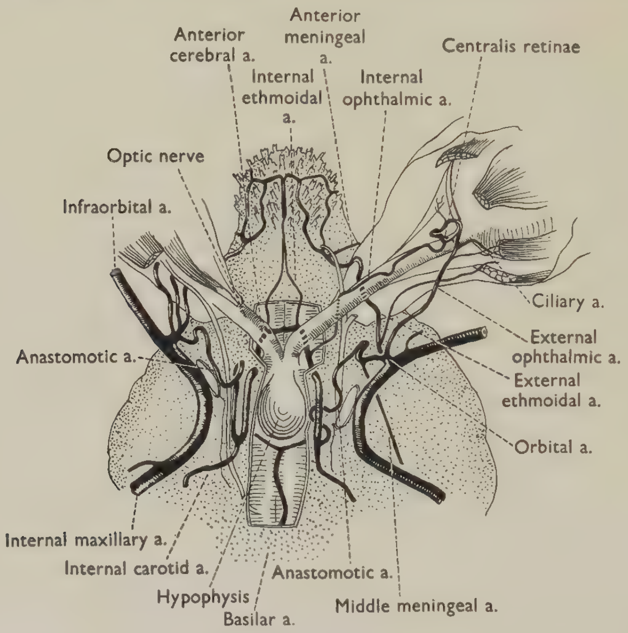


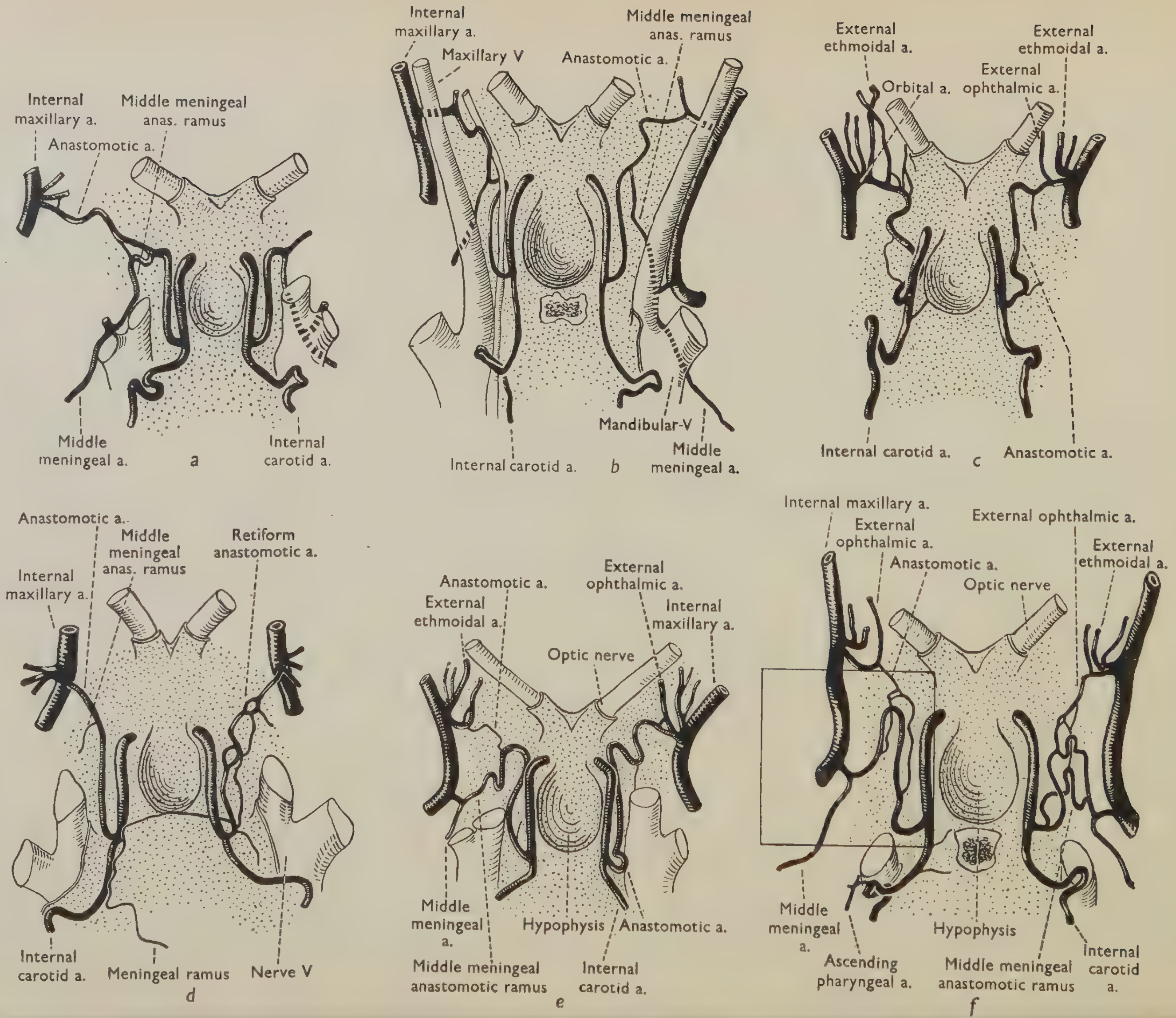
Fig. 4. Dissection of the brain and orbit, with entire cranium removed, to demonstrate the anastomoses between arteries of the orbital complex and intracranial vessels. Ventral aspect.

The third branch of the orbital artery which effects an anastomosis through the cranium is the ethmoidal artery. Ellenberger & Baum supposed this vessel to be homologous with the posterior ethmoidal artery of human anatomy, but Davis & Story (1943) have shown that in the dog the vessel supplies both the anterior and posterior groups of ethmoidal cells. Following the nomenclature of the latter authors it is here called the *external ethmoidal artery*, and the branch of the anterior cerebral artery with which it anastomoses the *internal ethmoidal artery*. Ellenberger & Baum had observed this vessel and named it the anterior ethmoidal artery; it appears, however, that this vessel is peculiar to the carnivores and has no homologue in man.

It may be noted that in the cat the orbital artery is not formed, but Davis & Story retain the name 'orbital complex' for the group of vessels which arises from the external rete. It is sometimes unformed in the dog, too, when the arteries of the complex arise directly from the internal maxillary artery. Under these circumstances the anastomotic artery and the external ophthalmic artery frequently form a common trunk at their origin, and the external ethmoidal artery and muscle rami do likewise. These varying conditions are shown in Figs. 4–6.

The anastomotic artery. The anastomotic artery is the largest single anastomosis between intracranial and extracranial vessels. Only 1 or 2 mm. of its length are visible outside the cranium, and for the greater part of its course it runs within the cavernous sinus, or that extension of the cavernous sinus which protrudes through the orbital fissure to be joined by the orbital venous sinuses. In Figs. 1 and 4 the anastomotic artery is depicted as a simple vessel running between orbital and internal carotid arteries (a condition found in some specimens), but typically the anastomotic artery receives a second contributory vessel within the cavernous sinus. This is the middle meningeal anastomotic ramus. The dual origin of the anastomotic artery is shown in Fig. 5*a*, where the two vessels contributing to the anastomosis are of equal calibre, whilst that of their union is conspicuously larger. The condition most frequently found is that in which the middle meningeal ramus is smaller in calibre than the orbital ramus (Fig. 5*b*). During the careful dissection of fourteen heads this condition was noted on both sides in seven of them; moreover, in a cursory examination of the region in many other heads dissected for other purposes this condition appeared most frequently. The other seven heads all showed some different variation. Dog 366 (Fig. 5*c*) is a variant of one extreme. The middle meningeal anastomotic contribution was not found on either side, whilst the orbital anastomosis was a large vessel. Between this and the condition of equality found in dog D7 (Fig. 5*a*) are all gradations, as seen on the right side of dog 309 (Fig. 5*d*), for example, and the left side of dog 352 (Fig. 5*e*). The right side of dog 352, however, exhibits the other extreme, where the middle meningeal anastomosis dominates and the orbital anastomosis is but a vestige. Dog D5 (Fig. 5*f*) had this condition on both sides, in fact, the middle meningeal anastomosis on the left is a tortuous double vessel to each of which the orbital branch contributes a delicate anastomosis. The complex approaches a retiform structure, and on the left side of dog 309, too, the anastomotic artery was formed into an arterial rete (Fig. 5*d*). Two other observations seem worthy of note. In dog 341 the middle meningeal artery of the right side entered the cranium by a separate foramen spinosum, but despite this, the internal maxillary artery still gave rise to a small vessel in the alisphenoid canal, which, passing through the foramen ovale, ran to unite with the anastomotic artery. In dog 353 (Fig. 4) there was an anomalous condition on the left side, the middle meningeal artery itself arising from the anastomotic artery instead of contributing to it.

In the cat, as Davis & Story have shown, the anastomotic artery is the largest artery of supply to the circle of Willis, and the fact that it plays such an important role in supplying the cerebral structures in one family of the Carnivora, suggests that it may be of importance in other families in which it is present. The misnomer of ‘ophthalmic’ artery has, perhaps, tended to disguise its function. That the anastomotic artery does make a contribution to the cerebral circulation in the dog is substantiated by the simple morphological observation that the internal carotid


Internal maxillary a.
Middle meningeal anas. ramus
Anastomotic a.
Middle meningeal a.
a
Internal carotid a.
Internal maxillary a.
Maxillary V
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Middle meningeal anas. ramus
Mandibular-V
Internal carotid a.
b
Middle meningeal a.
External ethmoidal a.
Orbital a.
External ophthalmic a.
External ethmoidal a.
Internal carotid a.
c
Anastomotic a.
Anastomotic a.
Middle meningeal anas. ramus
Retiform anastomotic a.
Internal maxillary a.
Internal carotid a.
Meningeal ramus
Nerve V
d
Anastomotic a.
External ethmoidal a.
External ophthalmic a.
Internal maxillary a.
Optic nerve
Middle meningeal a.
Hypophysis
Anastomotic a.
Middle meningeal anastomotic ramus
Internal carotid a.
e
Internal maxillary a.
External ophthalmic a.
Anastomotic a.
Optic nerve
External ophthalmic a.
External ethmoidal a.
Middle meningeal a.
Ascending pharyngeal a.
Hypophysis
Middle meningeal anastomotic ramus
Internal carotid a.
f

artery is larger after its junction with the anastomosis than before it. Further, the acute experiments of Bouckaert & Heymans (1935) suggest that the anastomosis normally contributes to the internal carotid flow. They showed that the flow in the anastomotic artery was sufficient, when all other sources of blood supply to the brain had been cut off, to maintain the nervous centres in a fully active state; and they showed, in a chloralosed dog, that when the anastomotic artery was clamped, the pressure in the cephalic end of the internal carotid artery fell considerably, suggesting that the anastomotic artery had been contributing blood to the internal carotid field.

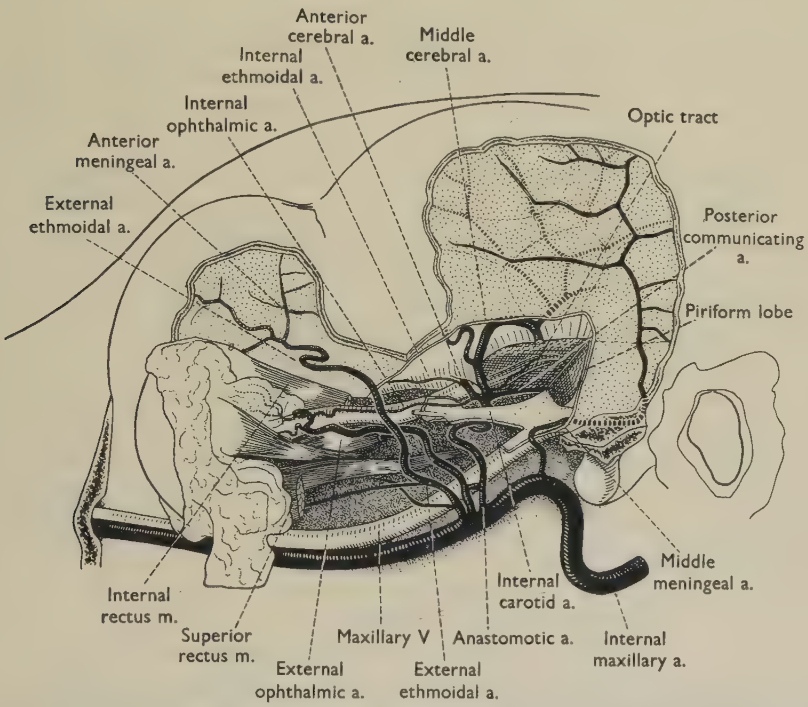

Fig. 6. Dissection of the orbit and base of the brain, lateral aspect, to demonstrate the ophthalmic anastomosis and the origin of the internal ophthalmic and internal ethmoidal arteries from the anterior cerebral artery beyond the division of the internal carotid.

The ophthalmic anastomosis. This anastomosis differs from those already described in that it is not one through which external carotid blood is likely to gain access to intracranial structures. Rather, it is a route by which the internal carotid may make a small contribution to the blood supply to the eye. The internal ophthalmic artery is a small vessel which arises from the anterior cerebral artery a short distance beyond the division of the internal carotid (Fig. 6). For the first third of its course it occupies a position lateral to the optic nerve and passes with it through the optic foramen into the orbit. Here it becomes closely applied to the nerve, which it crosses dorsally, to run along its antero-dorsal side. The external ophthalmic artery is one of the orbital complex of vessels and is usually associated with the anastomotic artery at its origin. It pierces the periorbita and passes between the retractor oculi muscles to assume a position close to the optic nerve. It becomes closely applied to the optic nerve a few millimetres from the bulbus and there anastomoses with the smaller internal ophthalmic artery. During this course it gives rise to rami to the retractor muscles, and a ciliary artery may be given off before the anastomosis with the internal ophthalmic artery is effected.

The central artery of the retina is difficult to detect by dissection, but by making serial sections through the region of the optic nerve and optic disc it has been possible to confirm Tandler's statement that the artery arises from the anastomosis formed by the internal and external ophthalmic arteries.

The ethmoidal anastomosis. For the greater part of its length outside the skull the external ethmoidal artery runs within the periorbita, from which it emerges to pass immediately through the ethmoidal foramen of the frontal bone and gain the ethmoidal fossa of the cranial cavity. Here it divides into three branches, the anterior meningeal artery and a dorsal and ventral ramus (Fig. 4). These latter vessels anastomose again more anteriorly, and the rete is joined by a contribution from the internal ethmoidal artery. The internal ethmoidal artery arises from the anterior cerebral artery distal to the origin of the internal ophthalmic artery and runs antero-medially until it meets its fellow from the other side (Figs. 4 and 6). The two vessels run forward close together, within the dura at the base of the falx cerebri, until they reach the cribriform plate where they diverge to anastomose with the vessels of their respective sides.

The ethmoidal anastomosis, like the ophthalmic anastomosis, is not one which forms a contributory channel to the cerebral blood supply. The external ethmoidal artery may carry blood of external carotid origin to the olfactory bulbs, but it is extremely unlikely, unless the normal circulation had been extensively interfered with, that blood carried by the external ethmoidal artery could return via the internal vessel to reach the circle of Willis.

DISCUSSION

A consideration of the carotid distribution from the point of view of the anastomoses between extracranial and intracranial arteries has a systematic, as well as a functional, justification. Davis & Story (1943) have observed that within different families of the Order Carnivora the carotid circulation shows divergent forms of specialization such that either the internal or external carotid arteries may become the main source of supply to the brain. In the cat the internal carotid artery has become completely vestigial and carries no blood, the ascending pharyngeal and internal maxillary arteries acting as alternative contributors to the circle of Willis. The opposite extreme is represented in the bears in which there are no interconnexions of importance between the intracranial and extracranial vessels. The dog occupies an intermediate position, but the present observations suggest that, so far as these vessels are concerned, it exhibits closer parallels with certain members of the Aeluroidea than Davis & Story had supposed.

These authors suggest that the primitive condition of these vessels in the Carnivora was similar to that now found in a genus such as *Bassariscus* in which the anastomotic artery is a simple vessel running between the internal maxillary artery and the internal carotid. Their observations upon a number of orders led them to conclude that the anastomotic vessel was subject to two opposite evolutionary developments: 'In the Carnivora the anastomotic vessel then presents two opposite evolutionary trends: in the direction of complete suppression in the Arctoidea, and towards extraordinary elaboration in the Aeluroidea.' If this assertion is generally true, then the present observations suggest that the domestic dog, a member of the Superfamily Arctoidea, must be regarded as an exception. Although the form of the anastomotic artery may vary considerably from one individual to another, not only was it present in all the heads examined, but it was usually nearly as large as the internal carotid artery, and in some cases showed a retiform development comparable in complexity with the rete in the aeluroid civet cat, as depicted by Tandler (1899). Again, with respect to the middle meningeal anastomotic ramus, the situation in the dog would appear to be comparable to that typically found amongst the Aeluroidea. In the cat the anastomotic ramus of the middle meningeal is larger than the middle meningeal itself, so that in this species the latter vessel appears to arise as a branch of the former. The middle meningeal artery is thus involved in the general redistribution of carotid blood to reach the circle of Willis via the external carotid field. This change in the role of the middle meningeal artery is evident in the dog too, where the anastomotic ramus is frequently found to be the larger distributory vessel and the meningeal artery appears to arise from it. Thus the suggestion of Davis & Story that this anastomotic ramus of the meningeal artery may characterize the aeluroid carnivores also finds an exception in the dog.

All these developments to alter the pattern of cerebral blood supply reach their extreme condition in the domestic cat where the brain receives blood only from external carotid and vertebral sources. The fact that the carotid system in the dog shows similar, though less advanced, changes, suggests that the importance which is usually attached to the internal carotid artery as supply route to the brain is not commensurate with its functional role. And whilst it is clear that the contribution of carotid blood to the brain is at least as great as that from the vertebral arteries, it is partly the anastomotic arteries, and perhaps the occipital anastomoses, which keep the proportions so adjusted.

SUMMARY

1. The arterial supply to the dog's head has been examined in a series of injected specimens in order to demonstrate the arterial pathways by which the brain may receive a blood supply.

2. Five anastomoses are described: the occipital-vertebral anastomosis, the ascending pharyngeal-internal carotid anastomosis, and the three anastomoses between the internal maxillary artery and intracranial vessels—the anastomotic artery, the ophthalmic anastomosis, and the ethmoidal anastomosis.

3. Of these anastomoses the occipital-vertebral anastomosis and the anastomotic artery are considered to be important routes by which carotid blood might reach the brain. In particular the role of the anastomotic artery is discussed: it is a contributory vessel to the internal carotid flow. The various forms which this artery may assume are described.

4. The similarity between the carotid vascular pattern in the dog and the aeluroid carnivores is discussed.

It is a pleasure to record my thanks to Prof. E. B. Verney for his advice and encouragement throughout the course of this work.

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SOME DERIVATIVES OF THE FOREGUT VENOUS PLEXUS OF THE ALBINO RAT, WITH REFERENCE TO MAN

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The foregut venous plexus gives rise to pulmonary and bronchial veins in addition to the veins of the trachea, oesophagus and stomach. This was first indicated by Brown (1913) in his account of the development of the foregut venous plexus of the domestic cat and, according to this investigator, the cranial part of the foregut venous plexus drains into the anterior cardinal veins and their intersegmental tributaries. Caudal to the septum transversum it drains into the omphalo-mesenteric veins, and has two direct connexions with the heart: cranially the 'pulmonary tap' which is the stem of the future pulmonary vein, caudally the 'postcaval tap' which becomes the termination of the inferior vena cava. The existence of the latter was not confirmed and it will not be discussed further. The foregut plexus between the pulmonary tap and the gastro-oesophageal junction becomes the venous plexus of the caudal third of the oesophagus which connects the pulmonary, portal and systemic venous systems.

The veins of the foregut plexus arise *in situ* in the mesoderm surrounding the foregut as was demonstrated in the human embryo of 6–7 mm. (Streeter, 1945). Its connexions with the pulmonary, portal and systemic veins are a direct result of the continuity of the splanchnic and somatic mesoderm.

Squier (1916) found transitory connexions between the pulmonary vein and the veins of the gut in the early chick embryo. Buell (1922) investigated the development of the pulmonary vein in the chick, and concluded that this vein was a persisting longitudinal vessel of the cephalic part of the splanchnic plexus. Chang (1931) came to similar conclusions. The intimate connexion of the blood vessels of the gut and lungs is also indicated by the arterial pattern (Königstein, 1903; Chauveau, 1873).

In the adult the oesophageal veins have many connexions with the systemic veins of the thorax and neck and, at the gastro-oesophageal junction, with the portal venous system. Less well known are the connexions between the pulmonary veins and the veins of the mediastinum, including the veins of the oesophagus (Zuckerkandl, 1881). Previous to the publication of this excellent review most anatomists agreed with Reisseisen & Soemmering (1808), who maintained that connexions between the pulmonary and systemic veins were confined to the broncho-pulmonary anastomoses within the lungs. Albrecht (1731) had shown that there were connexions between the pulmonary and mediastinal veins outside the lung, but most anatomists still regarded such connexions as anomalies. They had come to believe that a complete separation of the pulmonary and systemic veins must exist in order to maintain proper oxidation of the blood. They accepted the broncho-pulmonary anastomoses on the ground that the bronchial arterial blood never became venous because of its proximity to the air in the lungs. Zuckerkandl (1881) established the existence of considerable communication between the two circulations as a normal feature, and concluded that the influx of venous vessels into arterial ones was not contrary to the laws of nature but against the preconceived ideas held concerning connexions between arterial and venous vessels. He showed that a good injection of the pulmonary veins would also fill the veins of the oesophagus, the aorta, the diaphragm, the pericardium and finally the ramifications of the portal vein. He showed also that the veins of the oesophagus connected, at the lung roots, with the bronchial and pulmonary veins and, at the gastro-oesophageal junction, with the portal vein via the left gastric vein. He concluded that this anastomotic complex of the pulmonary veins would easily be explained if it was known what sort of vascular net there was in the primary anlage and how the respiratory net was derived therefrom. Unfortunately embryology was not then advanced enough to offer a satisfactory answer to this question. The work of Brown (1913) fulfilled this requirement.

Gilbert & Villaret (1908) showed that, in the dog, an injection into the portal vein will enter the veins of the lung. The main route is via anastomoses between the portal vein and the renal veins and so into the azygos veins, thence via the bronchial veins into the lung. If this route is cut off a small quantity of injection mass still enters the lung. It arrives there via small veins on the ventral surface of the oesophagus which join the peri-pleural and peri-bronchial plexuses.

Konaschko (1929) confirmed the findings of Zuckerkandl and showed that, in man, the bronchial veins have long branches which run cranially and caudally with the vagus nerves and connect with the veins of the oesophagus. The bronchial veins constantly have connexions with the pulmonary veins at the lung root and connect, via the oesophageal veins, with the left gastric veins and the systemic veins.

MATERIALS AND METHODS

A series of forty-two rat embryos of known age were used in this investigation. Some of the embryos were injected with indian ink (Butler, 1950). Table 1 lists the embryos used. Eight uninjected human embryos from the collection in the School of Anatomy, Cambridge, were examined (see Table 2).

OBSERVATIONS

(1) *The rat*

Stage 1. *Embryos of* 11 *days* 10 *hr., greatest length* 3 *mm.*

The foregut, surrounded by a continuous layer of splanchnic mesoderm, is beginning to differentiate into its main components. Cranially, branchial pouches 1 to 3 are present, but the ultimobranchial body still has a common opening into the pharynx with the 3rd pouch. These embryos closely resemble the 26-somite rat embryo reconstructed by Rogers (1929). The arch arteries 1 to 3 are large, and a small 4th arch artery lies in the groove between the 3rd pouch and the ultimobranchial body. From the ventral aorta, immediately adjacent to the caudal aspect of the ventral end of the 4th arch artery, a small vessel arises and runs caudally alongside the ventral surface of the foregut. This is the ventral part of the 6th arch artery with

Table 1. *Rat embryos*

Serial no.	Length (mm.)	Age		Plane of section	Thickness (μ)
		(days	hr.)		
17	3	11	10	Tr.	8
17.1				Tr.	6
i 44	3	11	10	Tr.	15
44.1				Tr.	7
19	6	12	9½	Tr.	8
19.1				Sag.	6
39	4·5	12	12	Tr.	7
i 39.1				Sag.	100
i 39.2				Cor.	100
i 39.3				Tr.	100
i 36.1	5	12	14½	Tr.	60
i 30	7	13	9	Tr.	60
i 30.1				Tr.	60
20	7	13	10	Tr.	8
20.1				Sag.	8
i 20.2				Tr.	75
i 20.3				Tr.	75
i 20.4				Tr.	100
20.5				Tr.	7
15	10	14	10½	Sag.	8
15.1				Tr.	8
i 15.2				Tr.	75
28	10	14	15	Tr.	12
i 28.1				Tr.	100
i 28.2				Tr.	100
i 28.3				Tr.	100
28.4				Tr.	12
28.5				Sag.	15
27	11	15	8½	Tr.	12
i 27.1				Tr.	100
i 27.2				Tr.	100
i 27.3				Tr.	100
i 27.4				Tr.	100
33	15	17	9	Tr.	15
33.1				Tr.	15
i 33.2				Tr.	100
i 33.3				Tr.	100
i 33.4				Tr.	100–200
i 33.5				Tr.	150
i 52	25	19	3	Sag.	200
i 52.1	23			Tr.	200
35	16	19	9	Tr.	15

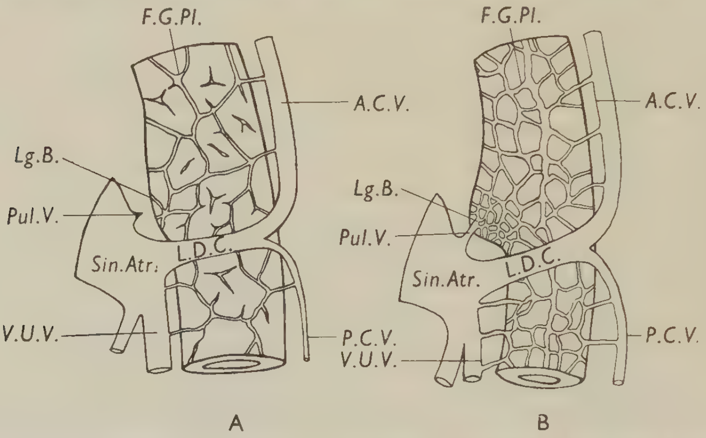
Embryos marked ‘i’ were injected with indian ink.

Table 2. *Human embryos*

Serial no.	Greatest length (mm.)	Plane of section	Thickness (μ)
H. 45	2·5	Tr.	5
—	8·0	Tr.	10
H. 102	9·0	Sag.	8
H. 250	12·25	Tr.	8
H. 78	13·5	Sag.	10
H. 53	16·5	Tr.	10
H. 209	30·0	Tr.	15
H. 109	50·0	Sag.	12

the caudally directed pulmonary artery. It is the main artery of supply to the vascular plexus which is developing in the mesoderm surrounding the foregut. The dorsal aortae, just caudal to the 4th arch artery, have three or four ventrally directed endothelial sprouts which are in the process of joining the foregut plexus.

Immediately caudal to the pharynx is the shallow tracheal groove and the commencing lung buds. The mesoderm is thicker and more deeply staining around the bifurcating tip of the tracheal groove. Formation of blood islands and their subsequent linking up to form a vascular plexus is taking place in the splanchnic mesoderm throughout the whole length of the gut. The plexus around the foregut, the post-branchial plexus of Huntington (1919), is the main object of this investigation and its arterial inflow has already been described. As yet the picture is incomplete, some of the vessels are still solid cords of angioblastic tissue, and much of it has yet to appear. It was difficult to be sure that the arteries from the dorsal and ventral aortae did actually join the patent parts of this developing plexus, but its venous connexions were easily seen and this suggests that some circulation had commenced. This plexus is best developed in the thicker mesoderm around the tracheal groove and lung buds.



Text-fig. 1. Diagrams illustrating the formation and routes of drainage of the foregut venous plexus in the rat (after Brown, 1913). A. 3 *mm. rat embryo, Stage* 1. The foregut plexus is not yet complete and is draining into the cardinal veins and the vitello-umbilical veins. The cardiac part of the pulmonary vein is seen as an endothelial outgrowth from the dorsal part of the sinu-atrial chamber and has not yet 'tapped' the foregut plexus. B. 4·5 *to* 6 *mm. rat embryo, Stage* 2. The stage of the pulmonary tap. The foregut plexus now drains into the cardinal, vitello-umbilical and pulmonary veins. The main longitudinal channels with the vagus nerves are well marked.

On each side of the foregut four or five distinct veins drain the plexus into the anterior and posterior cardinal veins, more particularly into the larger anterior cardinal veins. Caudal to the level of the ducts of Cuvier the foregut plexus drains into the vitello-umbilical veins, either directly or via the hepatic sinusoids (Text-fig. 1A).

The pulmonary vein develops in two parts and its cardiac end commences as an endothelial outgrowth from the dorsal wall of the sinu-atrial chamber. At this stage it is seen as a small conical proliferation of endothelium projecting into the dorsal mesocardium at the level of entry of the ducts of Cuvier and directed towards the bifurcation of the caudal extremity of the tracheal groove. It may show a small blind lumen opening into the sinu-atrial chamber. The peripheral part of the pulmonary vein will be formed from the pulmonary part of the foregut plexus which is developing *in situ* in the mesoderm around the lung buds. The rat embryo at this age closely resembles the 19-somite guinea-pig embryo described by Federow (1910).

The features of this stage are that the future pulmonary plexus is still inseparable from the remainder of the foregut plexus and that the cardiac end of the pulmonary vein is an endothelial bud on the dorsal wall of the sinu-atrial chamber.

Stage 2. *Embryos of* 12 *days* 9 *hr. to* 12 *days* 14 *hr., greatest length* 4·5–6·0 *mm.*

The relevant changes in the anatomy of the foregut are:

(1) The cranial end of the oesophagus is still in wide communication with the trachea since the tracheo-oesophageal septum is not yet well developed.

(2) The stomach can now be recognized as a fusiform dilatation of the foregut and it is commencing to rotate. The vagus nerves have reached its right and left surfaces. The part of the oesophagus between the tracheal bifurcation and the stomach is very short.

(3) The main bronchi are well developed and are surrounded by a thick layer of mesoderm. The longer right bronchus projects caudally into the mesoderm around the cardiac end of the stomach.

Each 6th arch artery now forms a complete arch, and it seems that the most cranial of the ventral branches from the dorsal aortae have been used to complete these arches. The remainder have disappeared. A large caudally directed pulmonary artery arises from each 6th arch artery and runs along the lateral aspect of the trachea. It is now the sole artery of supply to the post-branchial part of the foregut plexus. Caudal to the junction of oesophagus and stomach the foregut plexus is supplied by ventral branches of the dorsal aortae, the future coeliac axis.

The foregut vascular plexus is now well developed, particularly around the lung buds, but the gastric, oesophageal and pulmonary parts of the plexus are still inseparable. Its venous drainage is:

(1) A number of dorsally directed veins running ventro-lateral to the dorsal aortae into the large anterior cardinal veins.

(2) The short stretch of oesophagus between the level of the tracheal bifurcation and the stomach has one or two veins entering the post-cardinal veins close to the ducts of Cuvier.

(3) The perigastric part of the foregut plexus drains into the portal vein, which is now being formed from the vitello-umbilical veins, as well as having direct connexions with the liver sinusoids.

(4) The endothelial sprout from the dorsal wall of the sinu-atrial chamber has now penetrated the dorsal mesocardium, caudal to the transverse sinus of the pericardium, to join the foregut venous plexus caudal to the bifurcation of the trachea. It has become canalized, and blood from the ventral part of the foregut plexus, the future pulmonary plexus, drains directly into the heart. The opening of the single pulmonary vein is now well to the left of the midline and septum primum, opening into the left atrium (Text-fig. 1B).

The main longitudinal veins of the foregut plexus are close to the vagus nerves and, immediately caudal to the bronchi where the vagi lie close to the single pulmonary vein and its main tributaries, they enter this vein.

The arrangement of the venous drainage of the foregut plexus, at this stage, is identical with that shown in the 4·5 mm. cat embryo reconstructed by Brown (1913). It marks the end of the critical stage in the development of the pulmonary vein, i.e. the linking up of the cardiac outgrowth with the pulmonary part of the foregut plexus, and the foregut venous plexus has now established drainage into the pulmonary, portal and systemic venous systems.

Stage 3. *Embryos of* 13 *days* 9 *hr. and* 13 *days* 10 *hr., greatest length* 7·0 *mm.*

The stomach and lungs have grown rapidly in size but the oesophagus has grown very little, either in length or in thickness of its wall. The trachea has a thicker wall and wider lumen but is still short. These changes are reflected in the density of the foregut plexus in these regions: the perigastric and pulmonary plexuses are the densest, the peri-oesophageal plexus is the least dense and the peritracheal plexus is intermediate.

The endoderm lining of the oesophagus is still one cell thick and is surrounded by a zone of closely packed and deeply staining mesoderm which is its future muscle coat. Outside this is the peri-oesophageal venous plexus consisting of four to six longitudinal vessels united by frequent cross anastomoses. The main longitudinal veins are close to the vagus nerves. At the cardia the peri-oesophageal plexus is continuous with the perigastric plexus, particularly around the vagus nerves, and both plexuses communicate with the pulmonary venous plexus.

The venous drainage of the oesophagus is:

(1) Cranial to the tracheal bifurcation the peri-oesophageal and peritracheal plexuses are drained by a number of veins which run in a dorso-lateral direction to enter the anterior cardinal veins.

(2) At the level of the tracheal bifurcation one or two veins from the oesophagus join the terminal parts of the post-cardinal veins, which are now fully developed. The perisympathetic venous plexuses, which will replace the post-cardinal veins, are commencing to form (Butler, 1950).

(3) The perigastric plexus drains entirely into the portal vein and its direct connexions with the liver sinusoids have gone.

(4) The pulmonary vein now consists of main right and left branches which unite to form a single short trunk opening into the left atrium. Immediately caudal to the main bronchi the vagus nerves cross the right and left pulmonary veins, and their accompanying veins enter the dorsal aspect of the pulmonary veins. The main collecting area of these 'perivagal veins' is the oesophagus and the vagus nerves caudal to the bifurcation of the trachea. But since each perivagal plexus is continuous for the whole length of the oesophagus both also connect with the systemic veins and the left gastric vein. Thus the peri-oesophageal plexus of the caudal part of the oesophagus connects the pulmonary, portal and systemic venous systems.

Several small veins drain the venous plexus around the terminal part of the trachea and the commencement of the main bronchi into the pulmonary veins. These 'tracheo-bronchial veins' join the pulmonary veins close to or with the perivagal veins.

Caudal to the main bronchi a bridge of mesoderm connects the mesoderm around the oesophagus with that around the lungs. This will become the pulmonary ligament and it contains small veins connecting the peri-oesophageal and pulmonary venous plexus.

From this stage onwards it is convenient to divide the oesophagus into cranial and caudal segments separated by the plane through the tracheal bifurcation. These two segments differ in many ways:

(1) The main venous drainage of the cranial segment is into the systemic veins; the caudal segment drains mainly into the pulmonary and portal veins.

(2) The cranial segment is close to the vertebral column but the caudal segment is suspended by a meso-oesophagus which is longest at the level of the cardia. It contains veins only at its short cranial end.

(3) The mode of development and growth rate of the two segments differ considerably.

The foregut venous plexus is still a continuous system with drainage into the systemic, pulmonary and portal veins, but the proportions of the various parts of the plexus have altered as a result of the growth changes in the derivatives of the foregut.

Stage 4. *Embryos of* 14 *days* 11 *hr. and* 14 *days* 15 *hr., greatest length* 10 *mm. Embryos of* 15 *days* 9 *hr., greatest length* 11 *mm.*

The oesophagus has entered a period of rapid growth and differentiation. It has increased in length and diameter and the endoderm lining is now two to three cells thick. A submucous layer is present and it contains blood vessels.

The venous plexuses of the trachea and the oesophagus are mainly grouped around the vagus and recurrent laryngeal nerves, and the perisympathetic venous plexuses are now well developed. This may be called the 'stage of perineural venous plexuses'.

(1) *The cranial segment of the oesophagus.* The efferent veins from this part of the oesophagus now have two routes to the anterior cardinal veins: (*a*) the primitive route which lies ventral to the dorsal aortae and their derivatives; (*b*) new vessels, passing dorsal to the dorsal aortae, to join the perisympathetic venous plexuses which drain into the anterior cardinal veins by many small tributaries.

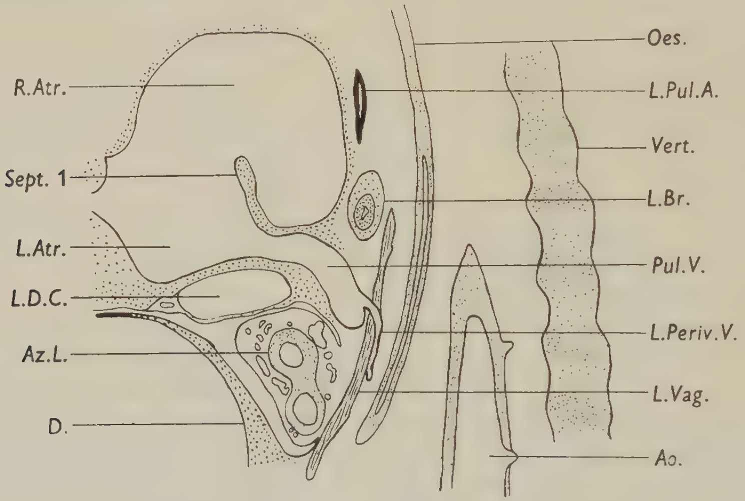
A continuous plexus of longitudinal veins, surrounding each vagus and recurrent laryngeal nerve, receives many veins from the oesophagus and trachea. At its cranial end the perirecurrent plexus is confluent with the veins surrounding the thyroid gland. Both perivagal and perirecurrent plexuses have many efferent veins to the anterior cardinal veins. On each side there is always a large vein draining both the perivagal and perirecurrent plexuses at the level of the origin of the recurrent laryngeal nerve. These veins pass under the right subclavian artery and the ductus arteriosus (Pl. 1, fig. 1). They are the inferior thyroid veins.

At the level of the bifurcation of the trachea one or two veins from the peri-oesophageal plexus join those parts of the post-cardinal veins which will persist as the termination of the left azygos vein and as the right superior intercostal vein (Butler, 1950).

(2) *The caudal segment of the oesophagus.* The perivagal veins of the caudal part of the oesophagus are well developed and join the extra-pulmonary parts of the right

and left pulmonary veins by a single trunk on each side (Text-fig. 2, Pl. 1, fig. 2). Close to their termination they receive the tracheo-bronchial veins. The pulmonary plexus can now be divided into pulmonary and bronchial components and veins draining the bronchial tree within the lung join the pulmonary veins both inside and outside the lung (Pl. 1, figs. 2 and 3).

There are no efferent veins from the caudal part of the oesophagus, in the region of the long meso-oesophagus, to the degenerating parts of the post-cardinal veins or the perisympathetic veins which are taking their place. The perivagal veins run with the vagus nerves on to the anterior and posterior surfaces of the stomach to form tributaries of the left gastric vein and so enter the portal vein or the terminal part of the splenic vein.



Text-fig. 2. Tracing of a sagittal section of a 10 mm., Stage 4, rat embryo, showing the longitudinal vein with the left vagus nerve. ×264.

The arterial supply of the cranial segment of the oesophagus is still from the pulmonary artery and the caudal segment is supplied by branches of the left gastric artery which ascend with the vagus nerves. There may be arteries from the internal mammary and superior thyroid arteries as well, but it is difficult to be certain.

Stage 5. *Embryos of* 17 *days* 9 *hr., greatest length* 15 *mm. Embryos of* 19 *days* 3 *hr. and* 19 *days* 9 *hr., greatest length* 16–25 *mm.*

The main change in the venous system is the emergence of the inferior thyroid veins from the plexus around the recurrent laryngeal nerves. Each vein is formed from the perirecurrent plexus and the vein which drains the combined perirecurrent and perivagal plexuses at the origin of the recurrent nerves. Thus the right inferior thyroid vein hooks under the proximal part of the right subclavian artery and the left hooks under the ductus arteriosus to join the right and left internal jugular veins respectively. In some specimens this long route is by-passed, and one of the efferent veins draining the perirecurrent plexus between the caudal pole of the thyroid gland and the cranial side of the subclavian arteries becomes the main channel. This is the inconstant middle thyroid vein (Greene, 1935) which is more frequent on the left than the right. All the thyroid veins receive oesophageal and tracheal veins.

Elsewhere the veins are unchanged except that the perivagal veins have become single trunks, one with each vagus nerve, opening into the extra-pulmonary part of the right and left pulmonary veins. Their connexions with the left gastric vein are much smaller.

The pulmonary artery no longer supplies the oesophagus, being replaced by the pericardio-mediastinal branches of the internal mammary arteries. Each has a main ascending branch, the inferior thyroid artery, and a small descending branch which anastomoses with an ascending branch of the left gastric artery.

Stage 6. *The adult rat*

Certain of the above findings were verified in the adult rat, in particular the longitudinal veins running with the vagus nerves and joining the left gastric vein to the pulmonary veins. They were demonstrated by tying the portal vein at its entry into the liver and placing a cannula in the superior mesenteric vein. Indian ink in reconstituted dried human blood plasma was then injected.

On each side of the oesophagus there is a longitudinal vein, running with the vagus nerve, and commencing as a branch of the left gastric vein. These veins receive tributaries from the peri-oesophageal and the perivagal venous plexuses, and terminate by joining the caudal branches of the pulmonary vein outside the hilum of the lung. The right vein is frequently larger than the left. Close to its termination the left vein has a small communicating branch to the left azygos vein. Similar veins were found in the guinea-pig but not in the rabbit, cat or dog. The observations of Gilbert & Villaret (1908) regarding communications between the portal and pulmonary veins via the superficial veins of the oesophagus, in the dog, were not confirmed.

(2) *Man*

Sections of a well-preserved human embryo, 2·5 mm. greatest length, show an early stage in the development of the foregut plexus and the pulmonary vein. Clumps of angioblastic cells in the mesoderm of the foregut are beginning to acquire cavities and unite to form a vascular plexus. The pulmonary vein is represented by a small solid endothelial outgrowth from the dorsal wall of the sinu-atrial chamber. It has no lumen and has not yet made contact with the foregut plexus. This embryo is, therefore, at an earlier stage of development than the previously described 3·0 mm. rat embryo (Pl. 1, fig. 4).

In an embryo of 8·0 mm. greatest length there is a well-developed venous plexus around the trachea and oesophagus and its main longitudinal vessels run with the vagus and recurrent laryngeal nerves. This plexus drains into the anterior and posterior cardinal veins and, at the gastro-oesophageal junction, it is confluent with the perigastric plexus which drains into the portal vein. The pulmonary vein stem, which grew out of the dorsal wall of the sinu-atrial chamber, now connects with the pulmonary part of the foregut plexus just caudal to the bifurcation of the lung bud. The single pulmonary vein appears to be relatively smaller than in the rat.

A similar arrangement is seen in embryos of 9·0 and 12·25 mm. greatest length Just caudal to the bifurcation of the trachea the vagus nerves pass close to the posterior aspect of the main right and left branches of the pulmonary veins and here the perivagal venous plexuses drain into the pulmonary veins. The peri-oesophageal plexus also drains into the cardinal and portal veins.

Embryos of 13·5 and 16·5 mm. greatest length clearly show the drainage of the caudal part of the perivagal plexuses into the extra-pulmonary part of the pulmonary veins. It was difficult to be certain that this was present in the 30 mm. embryo and these veins could not be found in the 50 mm. embryo.

DISCUSSION

The vascular plexus of the foregut is formed *in situ* from the angioblastic cells of the splanchnic mesoderm, and, because the splanchnic mesoderm forms a continuous layer over all the foregut and its derivatives, the vascular plexus is common to the gut, liver and lungs. At the root of the dorsal mesentery the splanchnic mesoderm is continuous with the somatic mesoderm and so the foregut plexus becomes continuous with the somatic vascular plexus. The foregut venous plexus has two primary routes of venous drainage:

(*a*) Ventrally into the vitello-umbilical veins.

(*b*) Dorsally into the cardinal veins.

The ventral part of the foregut plexus, surrounding the developing lungs, acquires a new and more direct venous drainage to the heart by the pulmonary vein.

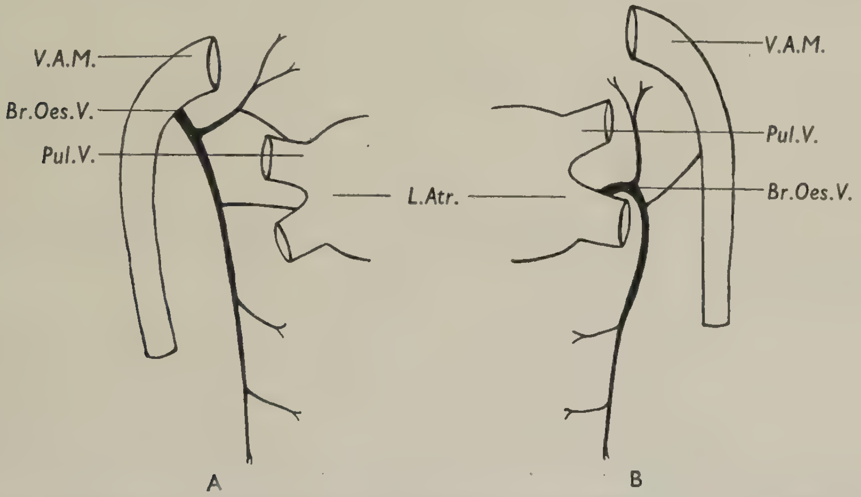
The 3 mm. rat embryo and the 2·5 mm. human embryo have no pulmonary vein and are, therefore, in the primary stage of the venous drainage of the foregut. By 4·5 mm. in the rat and 8 mm. in the human embryo the pulmonary vein has been formed and the three main parts of the venous system are present. This stage is seen in the 4·5 mm. cat embryo reconstructed by Brown (1913). The time of union of the two parts of the pulmonary vein in man can be more accurately stated from the date given by Streeter (1945). There is no pulmonary vein in the embryos of age group XIII (4–5 mm. long, estimated ovulation age 28 ± days). A single short pulmonary vein is present in embryos of age group XIV (6–7 mm. long, estimated ovulation age 28–30 days). In the rat, union of the two parts occurs between 11 and 12 days after copulation.

Brown (1913) believed that the portion of the foregut venous plexus surrounding the lung bud is pushed ventrally by growth of the lung bud and so comes into contact with the dorsal wall of the sinus venosus. Federow (1910) believed that the pulmonary vein grew directly from the dorsal wall of the sinus venosus as an endothelial bud which then divided into many branches in the lung. He did not believe that an indifferent capillary network could give rise to both arteries and veins. Flint (1907) had previously hinted at this mode of formation of the pulmonary venous system. Allied to this view is the one put forward by later investigators that the stem of the pulmonary vein grew out of the dorsal wall of the heart and united with that part of the foregut plexus which becomes the pulmonary plexus (Buell, 1922; Schornstein, 1931; Chang, 1931; Streeter, 1945; Auer, 1948).

In the material examined it appears that the pulmonary vein is a joint product from the dorsal sinu-atrial wall and the pulmonary part of the foregut venous plexus. The endothelial outgrowth from the heart forms the stem of the single pulmonary vein of the early embryo, but it was not ascertained how much of the adult pulmonary veins is formed from this outgrowth. Its mode of formation is 'an example of the affinity of one endothelial outgrowth for another' (Streeter, 1945), and it seems that the formation of the pulmonary vein is dependent upon a single outgrowth from the heart wall. Brown (1913) was probably misled as to the details of the mode of formation of the pulmonary vein because the youngest embryo in his series was one of 4·5 mm. length. His work is important because he recognized that the pulmonary venous plexus was simply a part of the foregut plexus which had attained a new route of entry into the heart but still retained connexions with the systemic and portal venous systems. Brown thus offered the advance in embryology required by Zuckerkandl (1881) to explain the occurrence of these connexions in the adult. It is in harmony with the work of Evans (1912) who showed how the blood vascular system develops from an indifferent capillary network which pervades the whole of the body of the embryo. Certain of these connexions, both normal and abnormal, are effected via the veins of the oesophagus and these will be discussed in some detail.

(1) *The perivagal veins*

A direct connexion between the portal and pulmonary veins, via the perivagal veins, normally exists in the adult rat and guinea-pig. Gilbert & Villaret (1908) reported the existence of small veins on the outer surface of the oesophagus which connected the portal and pulmonary veins in the dog, but this was not confirmed. Similar veins are found in the human embryo up to 16·5 mm. long. In the adult the venae comitantes of the vagus nerves connect the left gastric vein to the bronchial veins and these, in turn, have small connexions with the extra-pulmonary part of the pulmonary veins (Zuckerkandl, 1881). Thus, man and the rat differ only in the degree of separation of the pulmonary venous plexus from the other venous systems (Text-fig. 3).



Text-fig. 3. Diagram of the termination of the broncho-oesophageal vein in man (A) and the rat (B).

Among the reported cases of complete anomalous drainage of the pulmonary veins are a small number in which the pulmonary veins drain into the portal vein or into the ductus venosus close to its connexion with the portal vein (Brody, 1942; Edwards & DuShane, 1950). These abnormal veins run obliquely from left to right across the anterior surface of the oesophagus to pierce the diaphragm via the oesophageal hiatus. They then run along the lessser curve of the stomach, receiving the ascending part of the left gastric vein and becoming confluent with the descending part of the left gastric vein to join either the portal vein or the ductus venosus. These cases may be explained embryologically by assuming:

(*a*) Complete failure of the pulmonary vein outgrowth to join the foregut plexus.

(*b*) Utilization of the longitudinal channels which run with the left vagus nerve as an alternative outlet for the pulmonary blood flow in order to maintain the circulation. A further example of this anomaly will be described in detail in a subsequent paper.

(2) *Connexions between the pulmonary and mediastinal veins*

The mode of development of the foregut venous plexus offers an explanation of the small connexions between the pulmonary and mediastinal veins, particularly the oesophageal veins, which Zuckerkandl (1881) and Konaschko (1929) showed to be a feature of normal anatomy. The physiological consequence of these findings is that some venous blood is normally returned to the left atrium and this must be included among the reasons why the blood in the pulmonary vein is only 94–97 % saturated with oxygen. Konaschko (1929) attempted to estimate the percentage of venous blood entering the heart by these anastomoses by comparing their cross-sectional area with that of the pulmonary veins, concluding that 6–7 % of venous blood entered the heart by these routes. This method is fallacious since it assumes that the velocity and direction of the blood flow is the same in all the veins measured.

(3) *The bronchial veins*

The bronchial veins are formed from that part of the foregut venous plexus which surrounds the developing lung bud and they therefore communicate with the pulmonary, portal and systemic veins.

Lefort (1859) divided the pulmonary veins into:

(*a*) *True bronchial veins.* These veins drain the tissues of the lung root and the bronchial tree, as far as the second bifurcation, into the systemic veins.

(*b*) *Broncho-pulmonary veins.* These veins drain the remainder of the bronchial tree into the radicles of the pulmonary vein.

Zuckerkandl (1881) and Konaschko (1929) showed that the true bronchial veins communicate with pulmonary veins outside the lung roots.

In the developing rat embryo it is possible to follow the gradual separation of the bronchial veins from the pulmonary plexus, but even in the oldest embryo examined (19 days 9 hr.) there was no evidence of true bronchial veins. The venous drainage of the bronchi and the lowermost part of the trachea was by the broncho-pulmonary veins which the present writer has termed the tracheo-oesophageal veins, since they also drained the adjacent oesophagus. The lower oesophagus also drains into the pulmonary vein via the perivagal veins which frequently form a common trunk with the tracheo-oesophageal veins. This common vein, the broncho-oesophageal vein, is comparable to the vein formed in man by the union of the posterior bronchial vein and the oesophageal veins. Such a vein is found in many animals but it differs from the broncho-oesophageal vein of the rat in that it drains mainly into the systemic venous system (Text-fig. 3). Greene (1935) describes bronchial veins entering the superior vena cava in the adult rat, and Veerloop (1949) diagrammatically represents both true and broncho-pulmonary veins in the adult rat. In the present investigation these true bronchial veins could not be found in the adult rat or the late embryo. These facts suggest that the rat has a more complete broncho-pulmonary venous drainage than man, and that the degree of separation of the pulmonary, bronchial, portal and systemic venous systems varies from animal to animal.

(4) *The inferior thyroid vein*

The course of the inferior thyroid vessels in the rat is of considerable interest. Greene (1935) shows that the inferior thyroid arteries follow the course of the recurrent laryngeal nerves; on the right the artery loops under the subclavian artery, while on the left it loops under the arch of the aorta. The right inferior thyroid vein follows the same course as the artery, but Greene (1935) does not figure or mention the left vein. On both sides the superior thyroid veins run upwards and laterally in close proximity to the superior laryngeal nerves. An inconstant middle thyroid vein runs laterally from the lateral border of the lobe of the thyroid gland to join the internal jugular vein. In the embryo and sometimes in the adult the right inferior thyroid vein follows the course of the recurrent laryngeal nerve. Both inferior thyroid veins are present as well-defined channels before one of the efferent veins from the plexus around the recurrent nerves becomes enlarged to form the middle thyroid vein. It is possible that the growth of the neck and the recession of the subclavian artery and the arch of the aorta into the thorax lead to the obliteration of the inferior thyroid veins by stretching. This stretching will be more marked on the left side and the right inferior thyroid vein is more likely to persist. Man has no large vein corresponding to the inferior thyroid vein of the rat but it is represented by longitudinal anastomoses between the oesophageal veins which run close to the recurrent nerves to join the venous plexus on the lower poles of the thyroid gland.

SUMMARY

1. The development of the venous drainage of the foregut vascular plexus is described in rat and human embryos.

2. The primary drainage routes of this plexus are into the cardinal and vitello-umbilical veins.

3. The pulmonary venous plexus is developed from that part of the foregut plexus which surrounds the lung bud; it drains primarily into the systemic and portal venous systems. It then obtains a new route of drainage to the left atrium via the pulmonary vein.

4. The stem of the pulmonary vein is formed from an endothelial outgrowth from the dorsal wall of the sinu-atrial chamber which grows dorsally into the dorsal mesocardium and joins the pulmonary part of the foregut venous plexus. Thus the pulmonary venous plexus drains into the portal and systemic venous systems as well as directly into the heart.

5. In the rat the venous drainage of the oesophagus, caudal to the tracheal bifurcation, is into the extra-pulmonary part of the pulmonary vein via the perivagal veins. The caudal extremity of these veins joins the left gastric vein. Such veins are found in the human embryo up to 16·5 mm. greatest length. In the adult they are represented by the small connexions between the oesophageal veins and the pulmonary veins at the lung roots.

6. The bronchial veins are developed from the pulmonary part of the foregut plexus and drain primarily into the pulmonary vein, i.e. they are broncho-pulmonary veins. They may acquire systemic connexions to become true bronchial veins.

7. The degree of separation of the pulmonary, portal and systemic venous systems varies from animal to animal, and it is more complete in man than in the rat. In the adult animal there are always numerous connexions between the three venous systems and these may become enlarged under abnormal conditions.

8. The origin and course of the inferior thyroid veins is described in the rat and compared with man. Primarily the inferior thyroid vein follows the course of the recurrent laryngeal nerves.

My thanks are due to Prof. H. A. Harris for his advice and encouragement, to Messrs J. W. Cash and E. A. King for technical assistance, and to Messrs P. Harvey and A. E. Westwood for the photographs.

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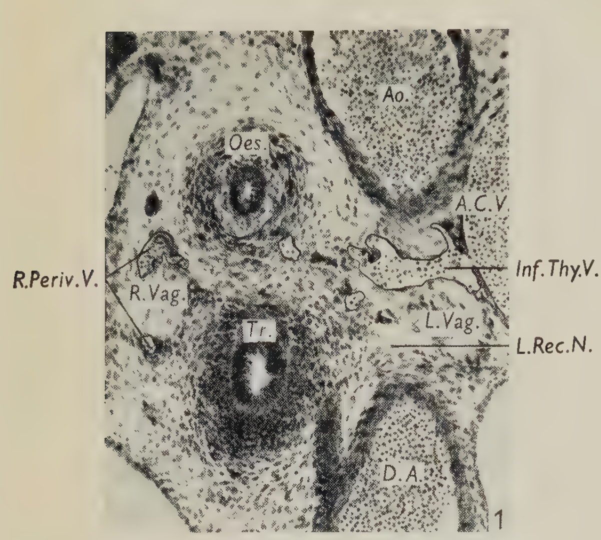

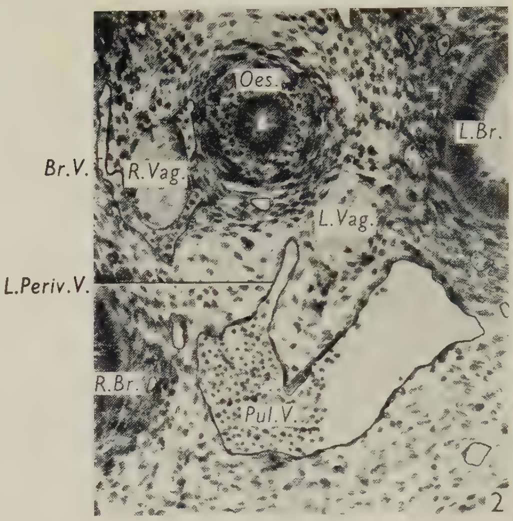

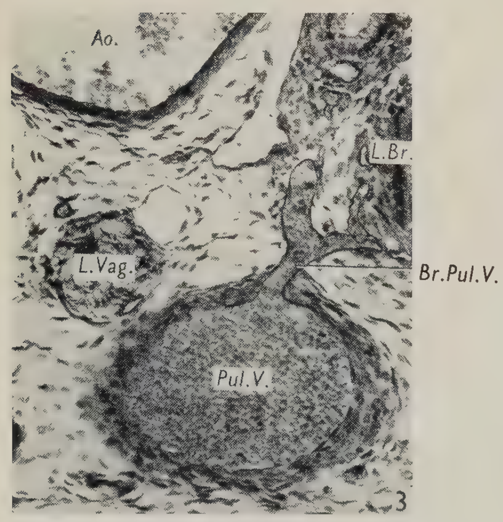

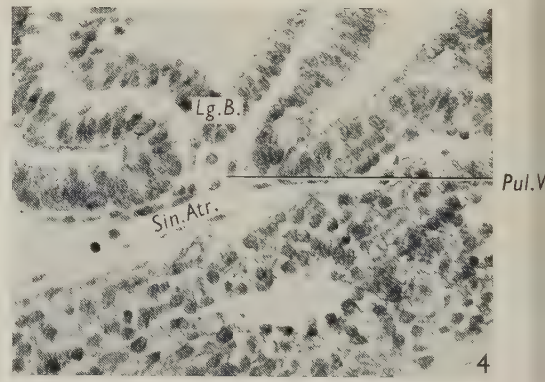

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EXPLANATION OF PLATE

For the sake of clarity the small veins in Figs. 1–3 have been outlined with indian ink.

Fig. 1. Photomicrograph of a transverse section of a 10 mm., Stage 4, rat embryo, showing: (1) The vascular plexus around the right vagus nerve. (2) The origin of the left recurrent laryngeal nerve and the vein which drains the venous plexus around this nerve into the anterior cardinal vein. This vein becomes the terminal part of the inferior thyroid vein. Haematoxylin and erythrosin. 12μ. × 200.

Fig. 2. Photomicrograph of a transverse section of a 10 mm., Stage 4, rat embryo, showing: (1) Entry of the left perivagal vein into the pulmonary vein. (2) A bronchial vein joining the right perivagal vein. Haematoxylin and erythrosin. 12μ. × 300.

Fig. 3. Photomicrograph of a transverse section of a 15 mm., Stage 5, rat embryo, to show a bronchopulmonary vein. Haematoxylin and erythrosin. 15μ. × 370.

Fig. 4. The pulmonary vein outgrowth from the dorsal wall of the sinu-atrial chamber of the heart. 2·5 mm. human embryo. Haematoxylin and erythrosin. 5μ. × 562.

KEY TO ABBREVIATIONS

A.C.V.	Anterior cardinal vein	*Oes.*	Oesophagus
Ao.	Aorta	*P.C.V.*	Posterior cardinal vein
R. and *L.Atr.*	Right and left atria	*R.* and *L.Periv.V.*	Right and left perivagal veins
Az.L.	Azygos lobe of lung	*L.Pul.A.*	Left pulmonary artery
R. and *L.Br.*	Right and left bronchi	*Pul. V.*	Pulmonary vein
Br.Oes.V.	Broncho-oesophageal vein	*L.Rec.N.*	Left recurrent laryngeal nerve
Br.Pul.V.	Broncho-pulmonary vein	*Sept.* 1	Septum primum
Br.V.	Bronchial vein	*Sin.Atr.*	Sinu-atrial chamber
D.	Diaphragm	*Tr.*	Trachea
D.A.	Ductus arteriosus	*R.* and *L.Vag.*	Right and left vagus nerves
L.D.C.	Left duct of Cuvier	*V.A.M.*	Vena azygos major
F.G.Pl.	Foregut venous plexus	*Vert.*	Vertebral column
Inf.Thy.V.	Inferior thyroid vein	*V.U.V.*	Vitello-umbilical veins
Lg.B.	Lung bud		

EXPERIMENTAL DEMONSTRATION OF CEREBELLAR CONNEXIONS FROM THE PERI-HYPOGLOSSAL NUCLEI (NUCLEUS INTERCALATUS, NUCLEUS PRAEPOSITUS HYPOGLOSSI AND NUCLEUS OF ROLLER) IN THE CAT

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INTRODUCTION

During an experimental study of the cerebello-petal connexions of the reticular formation of the medulla oblongata, it was observed that after lesions of the cerebellum retrograde cellular changes occurred in the nuclei surrounding the hypoglossal nucleus. Since a perusal of the literature revealed that almost nothing is known of the connexions of these nuclei, and since therefore no sound ideas on their functional significance can be set forth, it was felt worth while to pursue this study. Although it has so far not been possible to decide precisely to which part or parts of the cerebellum the fibres pass, the existence of cerebello-petal fibres from the peri-hypoglossal nuclear complex has been demonstrated. In the present communication the evidence upon which this statement is based will be presented, and observations on the reticulo-cerebellar connexions will be published separately. In addition, a description of the normal structure of the peri-hypoglossal nuclei in the cat will be given because no detailed analysis of their cellular architecture in the cat is available. This knowledge is necessary for a proper evaluation of the pathological alterations observed in the experimental animals.

The term 'peri-hypoglossal nuclei', when used in this paper, is meant to include those nuclei which are found in mammals immediately around the nucleus of the hypoglossal nerve. They are the following: (1) the nucleus intercalatus of Staderini; (2) the nucleus praepositus hypoglossi; (3) the nucleus of Roller. Other names have also been employed, and opinions differ with regard to certain points in the delimitation of the nuclei. Questions of nomenclature and observations of previous authors working on this subject may appropriately be considered when the observations made in the present study are discussed.

MATERIAL AND METHODS

The normal features of the peri-hypoglossal nuclei in the cat were studied in serial transverse sections stained with thionine (15μ thick).

For the experimental study of the cerebello-petal connexions of the nuclei, young kittens, 2–3 weeks old, were employed. With sterile precautions and under Nembutal (pento-barbital) anaesthesia, lesions were made in the cerebellum by means of dissection, suction or thermocoagulation. Four to six days later the animals were killed by exsanguination under chloroform anaesthesia, and the cerebellum and brain stem immediately removed. After fixation in 96 % alcohol the brain stem and cerebellum were embedded in paraffin and cut in transverse serial sections 15μ thick. The choice of very young animals and of the short post-operative period was made in the light of previous experience gained with the modified Gudden method (Brodal, 1940), and in the nuclei at present under investigation the retrograde cellular changes are very clear-cut with the procedure adopted. The presence of altered cells makes possible a far more precise determination of the origin of the fibres in question than the equivocal determination of a partial loss of cells in the nuclei which would have to be studied with longer survival times.

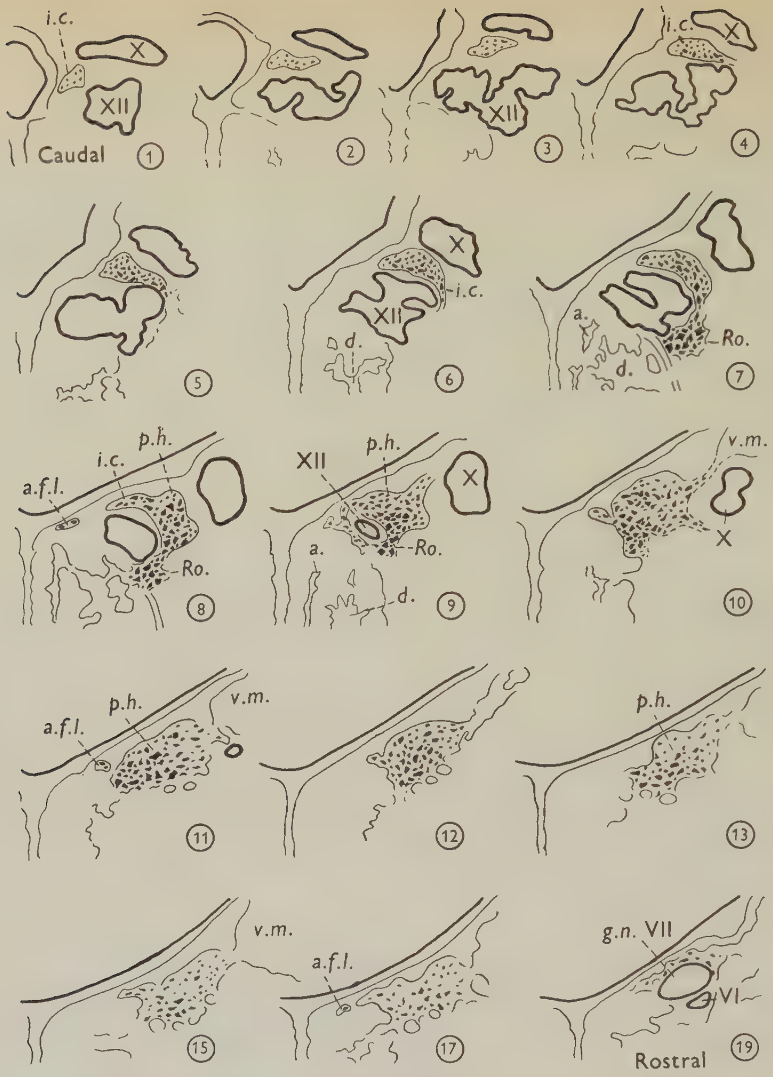
Supplementary and as yet provisional studies on some possible afferent connexions were made by means of the method of terminal degeneration. The modified Bielschowsky method of Glees (1946) was employed on adult cats for this purpose. The presence of terminal degeneration within the nuclei was sought after lesions of the midline structures of the cerebellum, hemisections of the brain stem at the level of the pons or after various lesions of the spinal cord.

NORMAL TOPOGRAPHY AND STRUCTURE OF THE PERI-HYPOGLOSSAL NUCLEI IN THE CAT

The description of the peri-hypoglossal nuclei to be given below is based on findings in cats 2–3 weeks old. In adult animals conditions are essentially similar, but the cells lie somewhat farther apart and contain more Nissl granules. The diagrams in Text-fig. 1 serve to illustrate the main topographical relations and cellular composition of the nuclei as seen at sixteen transverse levels in the medulla in a cat aged 15 days. The outlines were made by means of a projection apparatus, and the dots representing the cells filled in after direct examination under the microscope. The intervals between the sections drawn are equal except for the last three which are twice as large as the others. Pl. 1, fig. 1, shows drawings of the various types of cells composing the nuclei.

The nucleus intercalatus of Staderini is the first to appear when the region in question is traced caudo-cranially. It begins as a minute collection of small cells somewhat rostral to the caudal end of the hypoglossal nucleus, a little below the level of drawing 1 in Text-fig. 1. It is interposed between the hypoglossal nucleus ventrally and the dorsal motor (parasympathetic) nucleus of the vagus nerve dorsally, and retains this position for some distance. With the opening of the central canal the nucleus increases in size and lies between the hypoglossal nucleus ventromedially, the vagus nucleus dorso-laterally. Rostrally the nucleus intercalatus fuses imperceptibly with the nucleus praepositus. The transition appears to take place slightly below the level of the rostral end of the hypoglossal nucleus (8 in Text-fig. 1), since the cyto-architecture undergoes a change at this level (see below), and the nucleus increases markedly in size. In its rostral part the nucleus intercalatus shows some connexion ventrally with the nucleus of Roller (7 in Text-fig. 1). At more caudal levels its ventral or ventro-lateral border is sometimes indistinct (5 in Text-fig. 1), but otherwise it is everywhere clearly delimited from the hypoglossal and the vagal nuclei, although in places it is very closely related to the former. This is the case particularly at the ventral border of the nucleus intercalatus, where its cells may cover the dorso-lateral margin of the hypoglossal nucleus as a cap (5–7 in Text-fig. 1).

The majority of the cells composing the nucleus intercalatus are very small (Pl. 1, fig. 1*b*). They are round or oval and few of them are piriform or spindle-shaped.



Text-fig. 1. Drawings made by means of a projection apparatus of transverse Nissl-stained sections through the medulla oblongata of a cat 15 days old, showing the topography of the peri-hypoglossal nuclei. The intervals between the sections represented are equal, except for those between drawings 13, 15, 17 and 19 respectively, which are twice as large. The dots indicate the distribution and density of cells of different types. See also Pl. 1, fig. 1.

Abbreviations employed in this figure and in Text-figs. 2 and 3

a.	accessory group of paramedian reticular nucleus	*Ls.*	lesion
a.f.l.	anulus fasciculi longitudinalis posterioris of Ziehen	*ol.s.*	superior olive
c.r.	restiform body	*p.h.*	nucleus praepositus of Marburg
c.v.	ventral cochlear nucleus	*R.*	right
d.	dorsal group of paramedian reticular nucleus	*Ro.*	nucleus of Roller
flocc.	flocculus	*tr.sp.n.*V	spinal tract of trigeminal nerve
g.n. VII	genu of facial nerve	*v.l.*	lateral vestibular nucleus
i.c.	nucleus intercalatus of Staderini	*v.m.*	medial vestibular nucleus
L.	left	VI	nucleus of abducent nerve
		VII	nucleus of facial nerve
		X	dorsal motor nucleus of vagus nerve
		XII	nucleus of hypoglossal nerve

The relatively scanty cytoplasm is provided with fine, sparse Nissl-granules. The nucleus is clear and has a distinct nucleolus. In addition some medium-sized cells occur (Pl. 1, fig. 1*c*). Most of these are piriform, and their nuclei, which are a little larger than those of the small cells, frequently have a slightly eccentric position. The Nissl-granules are fine, but more abundant than in the cells of the smallest type.

While in the caudal part of the nucleus intercalatus only one or two of the medium-sized cells occur in each section, their relative number increases at more rostral levels (see Text-fig. 1). At the same time the density of cells increases. Near the rostral end of the nucleus (i.e. approaching the rostral end of the hypoglossal nucleus) an increasing number of larger multipolar cells appears. At this level the medium-sized cells dominate the cellular picture and are more numerous than the small cells (8 in Text-fig. 1). It appears appropriate from this level to name the rostral continuation of the cell mass of the nucleus intercalatus the nucleus praepositus hypoglossi.

The nucleus praepositus hypoglossi. While the nucleus praepositus is fairly well delimited in its caudal part, except for its continuity with the nucleus intercalatus, referred to above, its limits are rather diffuse at rostral levels. Its rostral end is here, somewhat arbitrarily, placed at the level of the caudal end of the nucleus of the abducent nerve, where the nucleus praepositus tapers dorso-medially to the genu of the facial nerve. Laterally it is more or less continuous with the medial vestibular nucleus (10–17 in Text-fig. 1) and ventrally with the cells of the reticular formation, but, particularly at rostral levels, it may be separated from the reticular formation by some fine bundles of longitudinally running fibres. The medial surface of the nucleus faces the fibres of the medial longitudinal fasciculus and is usually quite distinct. The relation of the nucleus praepositus to the nucleus of Roller is very intimate, the two nuclei fusing almost completely (7–9 in Text-fig. 1; see also Text-figs. 2 and 3). The relation to the hypoglossal nucleus is also of interest. It will be seen from Text-fig. 1 (drawings 8 and 9; see also Text-figs. 2 and 3) that where the nucleus intercalatus passes into the nucleus praepositus the latter surrounds the rostral tip of the hypoglossal nucleus and thus comes to occupy the position of the hypoglossal nucleus at more rostral levels (10 in Text-fig. 1).

The cells of the nucleus praepositus are of the small, medium-sized and larger types (Pl. 1, fig. 1*b*–*d*). The large cells are usually multipolar, the medium-sized most often piriform or spindle-shaped. The latter and the small cells are similar to those in the nucleus intercalatus. The density of the cells in the nucleus praepositus decreases gradually in a caudo-rostral direction from the level of drawing 13. As will be seen from Text-fig. 1, the cyto-architecture of the nucleus varies somewhat at different levels. As a general rule the cells composing its ventral part are larger than those in its dorsal part. Large cells are particularly abundant ventrally in its caudal third, where the nucleus establishes contact with the nucleus of Roller, which contains a great number of similar cells. Rostral to the level of drawing 15 in Text-fig. 1 cells of the largest type are scarcely encountered, but here also the ventral part of the nucleus contains the largest of the cells present, which are of the medium-sized type.

Even if certain areas of the nucleus praepositus are composed predominantly of larger or medium-sized cells and others of predominantly small cells, these differences are not distinct enough to justify a finer subdivision of the nucleus. From a topographical point of view the most cranial part of the nucleus praepositus might be called (in accordance with some previous authors) the *nucleus supragenualis nervi facialis*. However, no line of demarcation can be drawn between this and the rest of the nucleus praepositus. The rostral part of it (n. supragen. n. VII) is very loose in texture, and contains a few medium-sized and some small nerve cells.

The nucleus of Roller is not very clearly defined in the cat. However, at the ventral aspect of the rostral third of the hypoglossal nucleus fairly definite cell aggregations can be recognized. Most of the cells are situated lateral to the hypoglossal root fibres (7–9 in Text-fig. 1; see also Text-figs. 2 and 3) and are multipolar and rather large, but medium-sized and small cells also occur (Pl. 1, fig. 1*e*). These cells do not form a compact nucleus, but more massive parts alternate with regions in which only a few cells of the same types are found on the ventral surface of the hypoglossal nucleus. These groups, which are considered here to represent the nucleus of Roller, are frequently connected dorsolaterally by a cell strand with the nucleus intercalatus (7 in Text-fig. 1). At more rostral levels a similar connexion with the nucleus praepositus is even more convincing (8–9 in Text-fig. 1). The cell groups interpreted here as the nucleus of Roller are present also for some distance rostral to the hypoglossal nucleus where they appear as a ventral protrusion from the nucleus praepositus (10 in Text-fig. 1). This indication of a close relationship between the nucleus of Roller and the nucleus praepositus is emphasized by the similarity of the cells composing the parts of the nuclei in question.

Ventrally the nucleus of Roller is only indistinctly separated from the reticular formation. However, particularly along its rostral part, a certain separation from the reticular formation is established by some fine longitudinal fibre bundles, the same bundles, which, as mentioned above, are found ventral to the nucleus praepositus. The presence of these fibres makes the connexion between the nucleus of Roller and the nucleus praepositus more impressive.

While the nucleus of Roller can thus be recognized as a rather well-defined unit only where it lies ventral to the rostral third of the hypoglossal nucleus, in some cases similar smaller collections of cells are found ventral to the hypoglossal nucleus at more caudal levels. This, however, is not always so in normal material, an observation in keeping with the fact that the nucleus of Roller in the restricted sense presents considerable individual variations.

In the cat no nuclear group can be identified which might reasonably correspond to the *nucleus paramedianus dorsalis* of Jacobsohn, described in man (see discussion). The only cell complex, in addition to those already mentioned, which may deserve attention as a fairly distinct group is a tiny collection of medium-sized cells forming a sort of cap on the dorsal aspect of the medial longitudinal fasciculus. It is very variable, present as a rule only in a few sections, and medial to the nucleus praepositus (8, 11 and 17 in Text-fig. 1). It sometimes shows a connexion with the latter, but in any section seldom consists of more than two to five cells. This cell group has been identified with the *anulus fasciculi longitudinalis posterioris*, described in man by Ziehen (1934).

EXPERIMENTAL FINDINGS

In the young animals employed in the present study characteristic changes are observed in the cells of all the peri-hypoglossal nuclei 4–6 days after extensive lesions of the cerebellum. These changes are most clearly seen in the large cells, containing many Nissl-granules, but in the main they are similar in the smaller ones. In Pl. 1, fig. 1, drawings of representative specimens of altered cells of different types are reproduced. As will be seen from this figure and from the photographs in Pl. 1, figs. 2–5, there is a tigrolysis which affects almost the entire cytoplasm of the cell, leaving only a narrow zone with fine Nissl-granules along its periphery, thus indicating that the tigrolysis begins centrally. The nucleus is constantly found close to the periphery of the cell, frequently without any visible zone of cytoplasm between it and the cell border. In cells which are much affected, the nuclear membrane appears to disintegrate and the cytoplasm loses its milky appearance and becomes vacuolated. On account of the varying size of the cells it is impossible to decide whether the changes described are accompanied by any change in cell volume. Where many nerve cells are affected, astrocytes and microglial cells are increased in number and appear more active.

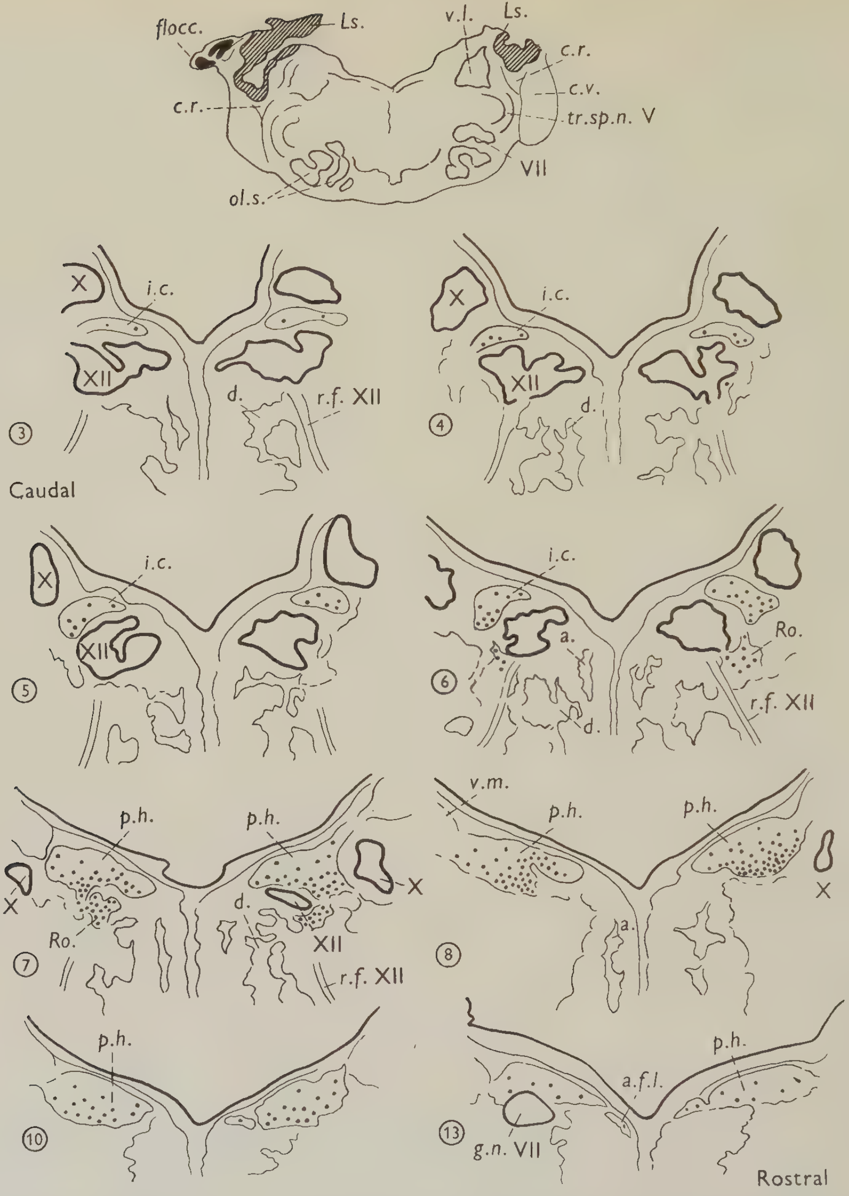
Only cells presenting the features mentioned have been considered as definitely pathological. When present there are usually interspersed between them others which show a diffuse tigrolysis but lack the homogenous appearance of the cytoplasm. Their nuclei are situated somewhat peripherally, but not extremely so as in the cells shown in Pl. 1, fig. 1. Probably they are cells in an earlier stage of change, but since somewhat similar cells may be seen in normal animals they have not been considered pathological.

The cellular changes observed are of the same type as those seen in other instances following transection of axons, the so-called retrograde cellular changes. For comparison a cell from the hypoglossal nucleus is included in Pl. 1, fig. 1. This cell, from a cat 21 days old at operation and killed 5 days later, shows the changes following transection of the root fibres of the hypoglossal nerve. The occurrence in the peri-hypoglossal nuclei of cells presenting similar changes after lesions of the cerebellum is taken to indicate that these cells project on to the cerebellum.

Cat O 93. Age at operation 15 *days; survival period* 5 *days* (Text-fig. 2, and Pl. 1, figs. 2–4).

After exposure of the caudal half of the cerebellum an attempt was made to remove the entire cerebellum by suction and blunt dissection. Serial sections show that this has almost been achieved; only small remnants of both flocculi and of the left paraflocculus are left. Probably the fibres to these parts have also been destroyed, since there is a necrosis extending for some distance into both restiform bodies and both brachia pontis (hatched in Text-fig. 2).

In the nuclei of the peri-hypoglossal complex cells showing typical retrograde changes are present, but they are not uniformly distributed, as will be seen from the diagram in Text-fig. 2. In the caudal part of the nucleus intercalatus cells that have undergone definite changes are rare. In its rostral half a moderate number of medium-sized and small cells present the typical picture of retrograde changes (Pl. 1, fig. 2). In its extreme rostral part (6 in Text-fig. 2) in which larger multipolar cells are present in increasing number a considerable proportion of these, as well as of the medium-sized piriform cells, are changed.



Text-fig. 2. Semi-diagrammatic representation of the findings in cat O 93 with an almost total cerebellar removal. Above, a drawing showing the remnants left of the cerebellum. Hatchings indicate partially destroyed tissue. Below, a series of drawings through the peri-hypoglossal nuclei. Dots indicate cells showing retrograde changes, the density giving an approximate impression of the distribution and relative number of changed cells. The level of the sections reproduced will be seen from their numbers, no. 1 (not included) passing through the most caudal part of the complex, no. 13 through its rostral end. For abbreviations see legend to Text-fig. 1.

The intensity of the changes increases somewhat, and reaches its maximum in that part of the nucleus praepositus which is particularly rich in large cells (Pl. 1, fig. 3, 8 in Text-fig. 2).

In the nucleus of Roller the majority of cells are altered (Pl. 1, fig. 4). Scattered changed cells are also found in the anulus of the medial longitudinal fasciculus.

Following a practically total removal of the cerebellum cells presenting retrograde changes are thus found in the nucleus intercalatus, the nucleus praepositus and the nucleus of Roller. Other experiments confirm these findings and give some additional information.

Cat O 84. *Age at operation* 14 *days; survival period* 5 *days* (Text-fig. 3)

The lesion is bilateral, but chiefly affects the right half of the cerebellum. From Text-fig. 3 it will be seen that certain parts have been destroyed (black), while others are partially damaged (hatched). On the right side the knife has passed so far ventrally as to cut a considerable number of the fibres of the restiform body where they fan out just after reaching the white matter of the cerebellum. The areas of cerebellar cortex to which these cut fibres pass are stippled (the determination of these areas was facilitated by a comparison with the alterations in the inferior olive, since the topography of the olivo-cerebellar localization is known from previous studies).

In the peri-hypoglossal nuclei cells of all types show retrograde changes. Their number is clearly smaller than in the preceding series (cat O 93), but their distribution is essentially similar (Text-fig. 3). The changes are bilateral, but they are more marked on the right side than on the left.

In this experiment, as well as in some others with extensive lesions of the cerebellum (e.g. cats O 91 and O 94), the retrograde cellular changes within the peri-hypoglossal nuclei are of the same type as in the first case described. Furthermore, the distribution of altered cells is similar. With asymmetrical lesions, the peri-hypoglossal nuclei present the most marked changes on the side of the most extensive cerebellar lesion. This might be explained if the peri-hypoglossal fibres to the cerebellum are either exclusively uncrossed, or if they are crossed as well as uncrossed, the uncrossed ones being more numerous. The following experiment gives information on this point.

Cat O 54. *Age at operation* 21 *days; survival period* 5 *days* (Pl. 1, fig. 5)

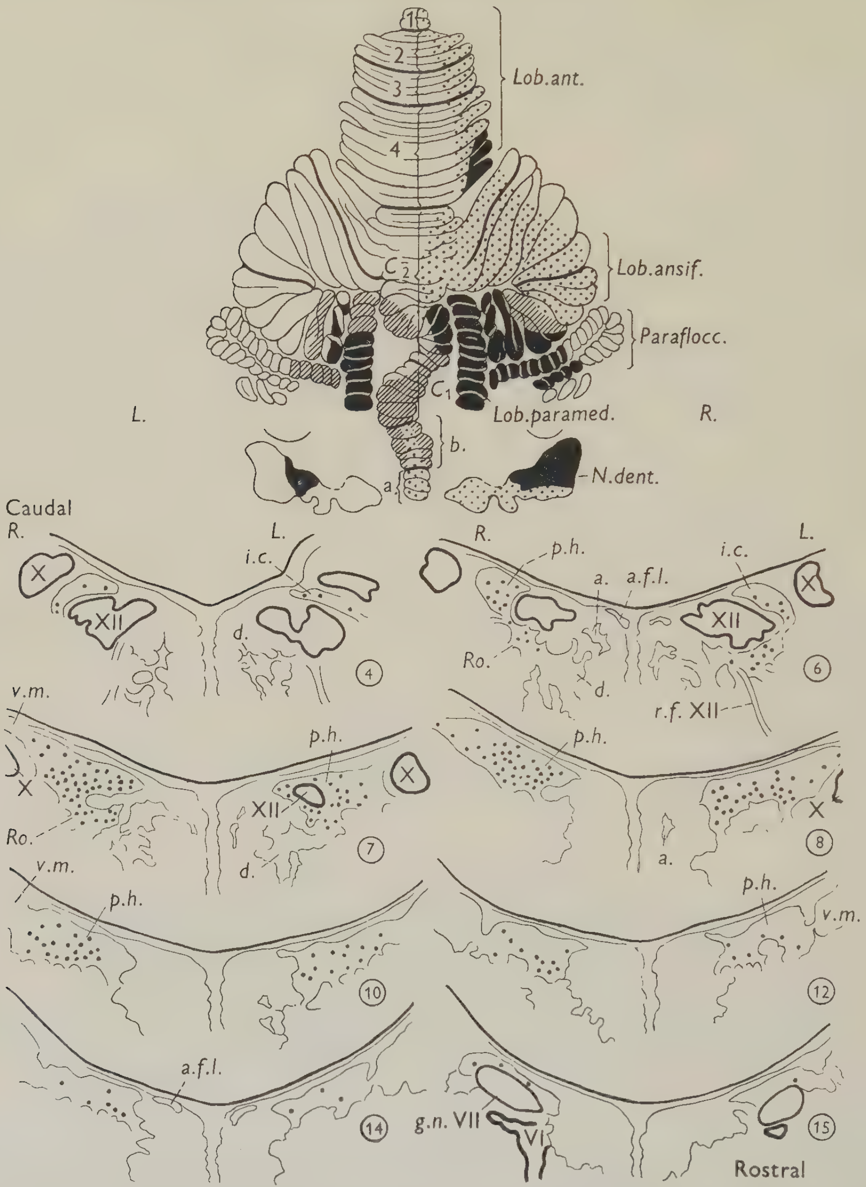
The right brachium pontis has been completely, and the right restiform body almost completely, transected. There is some accidental damage to the superficial aspect of the right side of the medulla, injuring chiefly the spinal tract of the fifth nerve.

Again the peri-hypoglossal nuclei show retrograde changes on both sides with a distribution corresponding to that observed in the other cases. The changes are somewhat more intense in the nuclei of the right side than in those of the left.

Since in this case practically all afferent fibres to the cerebellum have been interrupted on one side only, the occurrence of bilateral changes in the peri-hypoglossal nuclei demonstrates that their connexion with the cerebellum is crossed as well as uncrossed. From a comparison of the changes of the two sides it appears that the proportion of crossed to uncrossed fibres is approximately as 1 to 1·5–2.

Terminal degeneration within the peri-hypoglossal nuclei

Five days after extensive lesions of the brain stem at the pontine level abundant and typical terminal degeneration was observed in the peri-hypoglossal nuclei (adult cats, Glees's method). Particularly in the nucleus praepositus, numerous degenerating terminal boutons and fine degenerating terminal fibres were found (Pl. 1, fig. 6), in addition to some coarser degenerating fibres seen to enter from the



Text-fig. 3. Semi-diagrammatic representation of the findings in cat O 84. Above a diagram of the cerebellum unfolded into one plane. Black: areas totally destroyed. Hatching: partially damaged areas. Stippled: areas the fibres to which have been cut. Below a series of drawings through the peri-hypoglossal nuclei arranged as in Text-fig. 2. For abbreviations see legend to Text-fig. 1.

white matter close to the midline. Thus fibres descending in the brain stem must terminate in the peri-hypoglossal nuclei. This preliminary finding is of some interest, although further studies are needed to decide from what structures these fibres take origin and to establish their exact course and their exact pattern of termination within the peri-hypoglossal nuclei.

In a cat in which a lesion of the posterior vermis of the cerebellum (including both nuclei fastigii) had been made 5 days before sacrifice the peri-hypoglossal nuclei were searched for signs of terminal degeneration. Sparse and not quite conclusive changes are present in the nucleus intercalatus. The findings are difficult to evaluate, and since one case only has been studied the projection of fibres from the cerebellum to the peri-hypoglossal nuclei cannot be considered as proved although their presence may be suggested.

Following lesions of the spinal cord terminal degeneration was seen in the peri-hypoglossal nuclei in several cases. However, although unequivocal, the degeneration is far less, even with lesions of the highest cervical segments, than that which follows interruption of descending fibres.

DISCUSSION

A perusal of the pertinent literature reveals considerable confusion with regard to the designations employed for the various cellular groups surrounding the hypoglossal nucleus. This is probably due, at least to some extent, to the fact that these nuclei vary in different mammals and that there are considerable individual variations even in the same species. This has been pointed out by Jermulowicz (1934), among others, and is also evident in the present material. As long as knowledge of their fibre connexions is inadequate, the delimitation of the various nuclei must be based on topographical, cyto-architectural and comparative-anatomical criteria. The demonstration in this paper that all these cellular groups send fibres to the cerebellum emphasizes their close functional relationship, and serves to minimize the importance of a detailed separation into smaller cell groups. The subdivision of the nuclei employed in this paper, has therefore taken into account only the more conspicuous morphological features and before discussing the experimental findings some comment on the normal anatomy and terminology is appropriate. The nuclei have been most extensively studied in man.

(1) *The topography and delimitation of the peri-hypoglossal nuclei*

The nucleus intercalatus, described first in man by Staderini (1895), has been generally recognized by subsequent authors. Its position between the hypoglossal nucleus and the dorsal motor (parasympathetic) nucleus of the vagus is unequivocal. Among authors who have studied this nucleus in man may be mentioned Streeter (1903), Marburg (1904), Jacobsohn (1909), Weed (1914), Fuse (1914), Gagel & Bodechtel (1930), Jermulowicz (1934) and Ziehen (1934). Barnard (1940), in a comparative-anatomical study, found the nucleus intercalatus scarcely developed in the opossum but clearly present in the mouse, rat, rabbit, sheep, dog, cat, monkey and man. The continuity of the nucleus with the reticular formation, as described here, has been noted by several authors in various mammals and man.

Opinions differ only with regard to its rostral delimitation. Staderini originally applied the term nucleus intercalatus not only to the cellular group interposed between the hypoglossal and dorsal motor nucleus of the vagus, but to its rostral continuation as well (Staderini, 1938). This terminology was adopted by Streeter (1903) and Weed (1914), among others. The part extending rostral to the hypoglossal nucleus, however, has been considered a separate nucleus by most authors, first apparently by Marburg (1904) who called it the nucleus praepositus hypoglossi. Since this designation has gained general recognition it has been adopted in the present paper, the more readily since it is more appropriate from a descriptive point of view than other names employed. However, as was mentioned earlier no clear-cut boundary can be seen between the nucleus intercalatus in the restricted sense and the nucleus praepositus. According to available descriptions this appears to be the case not only in the cat but also in most other mammals including man.

In the cat, the cyto-architecture of the cell mass under consideration undergoes a change slightly caudal to the level of the rostral end of the hypoglossal nucleus. The chief feature of this change is an increased number of multipolar cells, medium to large in size. In the cat, therefore, it seems natural to place the transition between the nucleus intercalatus and the nucleus praepositus slightly caudal to the rostral end of the hypoglossal nucleus. In their atlas of the rabbit's brain stem Meessen & Olszewski (1949) appear to have done this, but it should be noted that the distinction is of importance chiefly for descriptive purposes.

The nucleus of Roller, described in man by Roller in 1881, has attracted the interest of several authors (Streeter, 1903; Marburg, 1904; Cajal, 1909; Jacobsohn, 1909; Weed, 1914; Nakamura, 1930; Gagel & Bodechtel, 1930; Goldin, 1934; Ziehen, 1934; and others). Jacobsohn (1909) called it the 'nucleus sympathicus sublingualis'. Goldin (1934) labels it 'nucleus comitans hypoglossi dorsalis secundus'. On the whole, subsequent descriptions are in accord with Roller's original report. The nucleus of Roller in man is found immediately ventral to the rostral half or third of the hypoglossal nucleus and medial to the root fibres of the hypoglossal nerve.

In mammals, generally the nucleus of Roller is, on the whole, less well developed and more difficult to identify than in man. Comparative studies have been made by Nakamura (1930) and Barnard (1940). Barnard found it well developed in the monkey and could identify it in the dog, cat, rabbit and sheep; he found it less distinct in the mouse, rat and shrew, but clear-cut in the opossum. In the rabbit the nucleus was also identified by Kimmel (1940) and by Meessen & Olszewski (1949). Berkelbach van der Sprenkel (1924) found it in *Erinaceus*. In monkeys the nucleus of Roller may be found medial to the root fibres of the hypoglossal nerve (Nakamura, 1930; Barnard, 1940), but in the other mammals in which it has been identified it lies lateral to these fibres. As we have seen, this is also the case in the cat, a point previously mentioned by Cajal (1909).

In keeping with the varying development of the nucleus of Roller in mammals, its cellular composition appears not to be the same throughout, as far as can be judged from the available literature. In man its cells are described as being small, having a diameter of some 15μ against the 60μ of the hypoglossal cells (Roller, Ziehen). Gagel & Bodechtel (1930), as well as other authors, stress the reticular appearance of the nucleus and mention its continuity with the nuclei interfasciculares nervi hypoglossi of Jacobsohn. The cellular strands connecting the nucleus of Roller with the nucleus praepositus have been noted also in the monkey by Barnard (1940).

There can be no doubt that the nucleus in the cat, described in the present study as the nucleus of Roller, corresponds with the nucleus of the same name in man. Its position agrees with that described for other mammals, and also the diffuseness of its border adjacent to the reticular substance. Its rostrodorsal connexion with the nucleus praepositus, which is very definite in the cat (see Text-fig. 1, drawings 8–9), has been noted also in man. With regard to its cells, it is interesting to note that Goldin (1934), who is one of the few authors to give a detailed account of the cells of the nucleus of Roller in man, distinguishes three types: (*a*) very small, rounded cells, with a comparatively large nucleus and scanty, finely granulated cytoplasm; (*b*) elongated cells; (*c*) large cells with a great mass of cytoplasm. This agrees well with the three different cell types present in the cat (Pl. 1, fig. 1*e*). An arrangement of its cells with their long axes parallel to the circumference of the nucleus, as described by Barnard (1940) in the dog, is not marked in the cat, as Cajal (1909) also found.

In addition to the well circumscribed nucleus of Roller, scattered collections of cells of a similar type are frequently found in the cat also at more caudal levels ventral to the hypoglossal nucleus. Such cell groups, present in some sections only, have been noted in other animals, and have been considered part of the nucleus of Roller. Since these cell groups in the cat react to cerebellar lesions in the same way as the cells of the nucleus of Roller, this view appears to be justified.

The nucleus praepositus. With regard to the rostral continuation of the nucleus intercalatus there is considerable confusion in the literature. In this paper the name nucleus praepositus nervi hypoglossi, proposed by Marburg (1904), is given to the cell column in question. From Marburg's atlas of the human brain it appears that his nucleus praepositus extends in a rostral direction to the level of the abducent nucleus. Marburg does not describe the cells of this nucleus, but its position as a longitudinal column occupying a position similar to that of the hypoglossal nucleus at more caudal levels, is clear, and justifies its name. Meessen & Olszewski (1940), in their atlas of the rabbit's rhombencephalon, use the name nucleus praepositus hypoglossi in the sense of Marburg. Since this terminology appears less equivocal than the name 'nucleus funiculi teretis', it has been adopted in the present paper, although the nucleus in question does not present a uniform appearance throughout in the cat. Since cells projecting on to the cerebellum are found even in its most rostral part, there seems to be little reason for applying different names to the various parts of this nuclear complex.

The discussion of the terminology of the nuclear complex, labelled here the nucleus praepositus, cannot, however, be abandoned without some references to the different terms found in the literature. Situated medial to his nucleus praepositus Marburg (1904), in man, distinguishes a slender longitudinal column of small cells, the nucleus funiculi teretis. The term nucleus funiculi or eminentiae teretis, unfortunately, has been used with different meanings. Thus Jacobsohn (1909) employs it for the cell group called nucleus praepositus by Marburg, while the small-celled column medial to the nucleus praepositus is termed nucleus paramedianus dorsalis. Jermulowicz (1934) uses the term in the sense of Jacobsohn, and claims that within the nucleus funiculi teretis three minor subdivisions, containing larger cells, should be separated as follows: caudally, a nucleus praepositus; rostrally a 'Kappenkern des Facialisknies' (nucleus supragenualis nervi facialis); and as a third complex the dorsal part of the anulus fasciculi longitudinalis posterioris of Ziehen, a group of medium-sized cells dorsal to the medial longitudinal fasciculus. Because of its inconsistent use, Ziehen (1934) argues that the term nucleus funiculi or eminentiae teretis should be deleted from the anatomical vocabulary. Instead he uses the designation 'raphe part of the nucleus paramedianus dorsalis' for the medial small-celled column, corresponding, it appears to Jacobsohn's nucleus paramedianus dorsalis, and the name 'ventricular part of the nucleus paramedianus dorsalis' for what appears to be Jacobsohn's nucleus funiculi teretis or Marburg's nucleus praepositus. Other variations in the use of the term nucleus funiculi teretis may also be encountered, but there is no need to review the confusing problem of nomenclature of the peri-hypoglossal nuclei more completely for this paper. Suffice it to say that according to most descriptions it is possible in man to distinguish a longitudinal column of small cells extending from the level of the hypoglossal nucleus to the level of the abducent nucleus (n. paramed. dors. of Jacobsohn, n. em. teretis of Marburg) and another column, situated just lateral to the first, composed of cells of different sizes but containing a certain proportion of medium-sized to larger cells (n. fun. teretis of Jacobsohn, n. praep. of Marburg). The larger cells in the latter are predominant at certain levels of its longitudinal extent, chiefly caudally and rostrally (n. praep. of Jermulowicz and n. supragen. n. fac. respectively).

In the cat no separate small-celled column can be seen which might correspond to the nucleus paramedianus dorsalis of Jacobsohn (1909). In his comparative-anatomical study Barnard (1940) illustrates in the medulla of the rabbit, sheep, dog, monkey and man a slender column of cells situated close to the midline, just beneath the floor of the fourth ventricle. He labels this column the nucleus eminentiae teretis and apparently uses this term in Marburg's sense (i.e. as equivalent to Jacobsohn's n. param. dors.). In his description, however, the nucleus is mentioned only in the dog, monkey and man, and in the dog it is said to be poorly represented. Since a similar nucleus cannot be found in the cat and is not mentioned or described by Meessen & Olszewski (1949) in the rabbit, it is possible that Barnard was mistaken with regard to the rabbit and the sheep. It appears that the nucleus paramedianus is a differentiation of the peri-hypoglossal grey matter which has appeared only late in phylogenesis. A nucleus praepositus (in the sense of Marburg) is mentioned by Barnard only in his account of monkey and man. Marburg (1931) emphasizes that the nucleus praepositus is more easily recognized the higher one proceeds in the phylogenetic scale, and that at the same time its continuity with the nucleus intercalatus becomes less marked.

It appears from the literature that the peri-hypoglossal nuclear complex becomes increasingly differentiated in higher mammals, reaching its peak in man. In the cat this differentiation has not yet proceeded to the formation of a nucleus paramedianus. Which cells of the nucleus praepositus (in the sense employed here), if any, correspond to this nucleus in monkey and man, it is impossible to decide. It is worth noticing, however, that the nucleus praepositus of the cat displays the same general topographical and cytological features as the corresponding structure in man. Thus, according to Jermulowicz (1934), in man the larger cells are at all levels concentrated in the ventral part of the nucleus. The same is the case in the cat, and is probably a feature of some functional importance. The concentration of larger cells at the caudal and to some extent at the rostral ends of the nucleus is also similar in the cat and in man. The rostral condensation might be referred to as the *nucleus supragenualis nervi facialis.* Its position close to the nucleus of the abducent nerve suggests a functional relationship similar to that which may exist between the caudal condensation and the hypoglossal nucleus. If this suggestion should be substantiated, it would be appropriate to separate the rostral condensation from the nucleus praepositus and to use a special name for it.

The anulus fasciculi longitudinalis posterioris of Ziehen (1934) is described by him in man at levels rostral to the nucleus of the abducent nerve. It more or less surrounds the fibres of the medial longitudinal fasciculus. Its cells are said to be larger than those of the nucleus paramedianus dorsalis, and the nucleus may be broken up into minor cell groups.

In the cat a few medium-sized cells seen at some levels medial to the nucleus praepositus resemble a little what Ziehen describes in man. However, in the cat these cell groups are found further caudally and they do not completely surround the fasciculus. Their relationship with cells of the raphe appears to be the same as in man. In spite of the differences mentioned, it was deemed appropriate to label the nuclear group the anulus of the medial longitudinal fasciculus, emphasis being laid on its close relation to that fibre bundle.

(2) *Fibre connexions of the peri-hypoglossal nuclei*

After extensive lesions of the cerebellum the changes in the cells of the peri-hypoglossal nuclei are those characteristic of the retrograde degeneration which occurs in nerve cells when the axon is transected. They are virtually identical with the changes seen in other cells known to project upon the cerebellum, e.g. the cells of the external cuneate nucleus, the lateral reticular nucleus and the pontine nuclei (Brodal, 1941, 1943; Brodal & Jansen 1946), and may therefore be taken as showing that the cells in question send their axons to the cerebellum.

In the experiments reported all cells of the peri-hypoglossal nuclei were never affected, not even when practically the entire cerebellum was removed. This seems to indicate that some send their axons to other regions of the brain, but another possibility must also be considered. It is well known from other instances that all the cells of a nucleus do not react with the same speed to damage to the axon (Brodal, 1939). Considering the short survival time employed in the present study it is, therefore, probable that some cells have not yet reached a sufficient degree of alteration to be recognized as changed. Experiments with total ablations of the cerebellum and a longer survival period would help to decide this question, but so far it has not been possible to keep such animals alive for more than 6 days. If only the cells seen to be definitely pathological are taken into account, it appears that at least the majority of the cells of the caudal third of the nucleus praepositus and of the nucleus of Roller project on to the cerebellum, while the rostral two-thirds of the nucleus praepositus contain a much smaller proportion of cells of this type.

With regard to the nucleus intercalatus the evaluation of this point is more difficult since the small cells, of which this nucleus is chiefly composed, are difficult to analyse with regard to cytological changes. However, a careful comparison of cells in the experimental animals with normal controls leaves no doubt that a certain number of the small cells in the experimental animals are pathological: they are rounded, the nucleus is found in the extreme periphery of the cell and almost no Nissl-granules are left (Pl. 1, figs. 1 and 5). It is impossible from the material available at present to decide whether many of the other cells are normal or in the early stages of retrograde change. Until further evidence is brought forward, it seems safe to conclude only that a certain proportion of the cells of the nucleus intercalatus project on to the cerebellum; the proportion is somewhat greater in the rostral than in the caudal levels of the nucleus.

Apart from the conclusion that a somewhat greater proportion of the connexions described run to the homolateral rather than to the contralateral half of the cerebellum, no suggestions can be offered with regard to the finer organization of this projection. Thus no conclusions can be drawn concerning the exact area within the cerebellum in which the fibres from the peri-hypoglossal nuclei terminate. Nor can it be decided whether fibres from different parts of the nuclear complex terminate in different areas. Experiments are being undertaken to elucidate these questions, but even without knowledge of these details the present observations are of interest since they demonstrate a connexion of the peri-hypoglossal nuclei which, as far as is known, has not been suggested before, and which may contribute to our understanding of the function of these peculiar nuclei.

As has been seen, the nucleus intercalatus fuses imperceptibly with the nucleus praepositus, and both these nuclei are connected by conspicuous cellular strands with the nucleus of Roller. These topographical features point to a functional relationship between the three nuclei, the existence of which is strongly emphasized by the demonstration of a common projection to the cerebellum. It is also of interest to note that the peri-hypoglossal nuclei fuse with other nuclei which have cerebellar connexions. Thus the nucleus praepositus is continuous with the medial vestibular nucleus, which, as has been verified in this material, sends fibres to the cerebellum. The nucleus of Roller establishes a cellular connexion with a particular group of cells of the reticular formation, situated just lateral to the raphe (labelled *d* in Text-figs. 1–3). This is one of three small reticular cell groups which project to the cerebellum and which have been termed collectively the nucleus reticularis paramedianus (Brodal, 1952). In addition, scattered cells along the course of the root fibres of the hypoglossal nerve (nuclei interfasciculares n. hypoglossi of Jacobsohn) behave in the same manner, so that here, as in so many other cases, topographical features reflect functional relationships.

The close topographical relation between the hypoglossal nucleus and the peri-hypoglossal nuclei naturally has been taken as an indication that the nuclei in question are of importance for the functions of the tongue. The demonstration of a quantitatively important projection from these nuclei to the cerebellum lends support to the contention, advocated by Du Bois (1929), that they are in some way concerned with the proprioceptive innervation of the tongue, and argues against the view that they are related to taste. The increasing development of these nuclei as the mammalian scale is ascended (Tagaki, 1925*b*; Kappers, Huber & Crosby 1936; Barnard, 1940) also points in the same direction.

The cerebellar connexions of the peri-hypoglossal nuclei described here appear at present to be their only definitely established efferent connexions. The original contention of Roller (1881) that the axons from his nucleus join the root fibres of the hypoglossal nerve, although supported by some authors, has not been verified by controlled experimental studies (see Barnard, 1940) or by careful analyses of normal material (Cajal, 1909; Ziehen, 1934; Kimmel, 1940; Boyd, 1941; and others). Nor have changes been observed in the other peri-hypoglossal nuclei following transection of the hypoglossal nerve (Tagaki, 1925*a*; Barnard, 1940). It appears likely that the cells of these nuclei are functionally related to those of the hypoglossal nucleus proper, but whether axons or collaterals from the former are among the fibres passing in great number between the peri-hypoglossal and the hypoglossal nuclei has not been experimentally verified, although Kimmel (1940) in normal rabbit material thought this to be so. Future studies must decide whether all or some of those cells of the peri-hypoglossal nuclei which apparently do not send their axons to the cerebellum project on to other parts of the brain, or whether they are to be considered as internuncial cells, serving for the reception of afferent impulses to the nuclei. It has been suggested (Marburg, 1931) that the larger cells of the peri-hypoglossal nuclei are 'motor', giving origin to efferent fibres, while the small cells are 'sensory', receiving impulses from afferent fibres and transmitting them to other cells of the nuclei. However, as has been seen, cells of all types project on to the cerebellum, so that the presence in the nuclei of cells of different sizes can scarcely be explained on this basis alone, although the efferents to the cerebellum may take their origin chiefly from those regions which are particularly rich in larger cells. A more probable explanation can be given for the presence of cells of different sizes. Since large cells have in general thicker axons than small ones, it may be presumed that the consequent differences in physiological properties, such as the height and duration of the action potential and the conduction velocity, are the functional correlates of the morphological differences. If future studies should demonstrate that afferents from the same source establish synaptical contact with cells of all types, this interpretation would be supported. That it is possible is shown by observations made in the lateral reticular nucleus (nucleus funiculi lateralis) in which large as well as small cells project on to the cerebellum (Brodal, 1943), and both types receive spinal afferents (Brodal, 1949). If there are true 'sensory' or better, internuncial cells, in the peri-hypoglossal nuclei, it appears that they must be sought chiefly in the nucleus intercalatus and the rostral two-thirds of the nucleus praepositus.

The present study suggests that the peri-hypoglossal nuclei when discharging must influence first and foremost the cerebellum (possibly particular regions of it) and probably the hypoglossal nucleus as well. The peri-hypoglossal nuclei themselves appear to be influenced by impulses from different sources. According to the findings reported here, an important contribution must be represented by fibres from higher levels descending in the brain stem. Whether some of these fibres pass via the fasciculus longitudinalis dorsalis of Schütz as described by Nakamura (1930), but not verified by Barnard (1940), cannot be decided from observations made in normal material. Other afferents appear to come from the vestibular nuclei, particularly the medial nucleus, by way of Fuse's fasciculus triangulo-intercalatus (Fuse, 1914; Tagaki, 1925*b*), but in his experimental study of the efferent connexions of the vestibular nuclei, Buchanan (1937) does not mention such fibres. Of particular interest is the question of possible cranial nerve afferents. In normal material from different mammals several authors have observed fibres of the vagus and glossopharyngeal nerves passing to the nucleus intercalatus (Du Bois, 1929; Barnard, 1940; Kimmel, 1940, 1941), to the nucleus praepositus (Fuse, 1914; Kimmel, 1941), and even to the nucleus of Roller (Kimmel, 1941, in the rabbit). In experimental studies with the Marchi method Du Bois (1929) found fibres of the vagus passing to the nucleus intercalatus, while Allen (1923) in the guinea-pig failed to do so. In a human pathological case Schwartz, Roulhac, Lam & O'Leary (1951) did not see them.

If the peri-hypoglossal nuclei are concerned in the regulation of the motor activities of the tongue, it would be reasonable to assume that they receive proprioceptive impulses from this organ. The problem of the proprioceptive innervation of the tongue is, however, far from clear. Widely diverging views on the presence and abundance of proprioceptive receptors in the tongue have been expressed in the literature (see, for example, Langworthy, 1924; Weddell, Harpman, Lambley & Young, 1940; Boyd, 1941), and the central course of possible proprioceptive fibres is uncertain. While some authors have observed a moderate number of ganglion cells along the hypoglossal nerve in different mammals and man (Tarkhan & Abd El-Malek, 1950), and suggest that at least some proprioceptive impulses may travel via this nerve, others have been unable to verify the existence of such cells (Carleton, 1938; Boyd, 1941), and Barron (1936) did not obtain action potentials from the hypoglossal nerve after deforming, stretching or burning the tongue in experimental animals. Boyd (1941) suggested that the cells of the so-called Froriep's ganglion on the hypoglossal nerve may have become incorporated in the first or second cervical dorsal root ganglion, and the demonstration that some fibres of the hypoglossal nerve degenerate after removal of these ganglia in the rabbit (Yee, Harrison & Corbin, 1939) supports this view. However, the available evidence relating to possible afferents in the hypoglossal nerve indicates that they are very few in number and that other pathways for proprioceptive impulses from the tongue must be sought for. The disputed fibres of the vagus and glossopharyngeal nerves to the nucleus intercalatus may perhaps be of this kind, as suggested by Du Bois (1929).

The assumption of a relation of the peri-hypoglossal nuclei to motor functions of the tongue is strengthened by Allen's (1927) observation of fibres from the nucleus fastigii to the nucleus intercalatus in Marchi preparations of the guinea-pig. If further studies should verify their existence, they would provide evidence for a closed circuit between the cerebellum and the peri-hypoglossal nuclei.

Evidence has been reported in this paper that fibres ascending from the spinal cord terminate in the peri-hypoglossal nuclei. This is surprising and the functional significance of such fibres is at present entirely obscure. There is little doubt, however, that investigations using the method of terminal degeneration will unravel many unexpected fibre connexions, at least as perplexing to our present-day conceptions of the organization of the nervous system as those just referred to. With regard to the peri-hypoglossal nuclei, we are as yet far from understanding their functional organization, which appears to be more complex than generally assumed. At present we can only state that they may be activated by impulses from numerous sources and that they have important relations with the cerebellum;

it may be assumed that they are probably involved in some way in the regulation of the motor functions of the tongue. Much work is needed to clear up the many obscure problems related to these small and peculiar nuclei. It is certain that they are functionally important, not least so in man.

SUMMARY

1. The topography and cellular architecture of the peri-hypoglossal nuclei in the cat are described. The nucleus of Roller, the nucleus intercalatus of Staderini and a nucleus praepositus in the sense of Marburg can be differentiated. The three nuclei are interconnected by cellular strands, and fuse also with certain parts of the reticular formation and the medial vestibular nucleus. The confusing terminology of the nuclei is briefly discussed.

2. After extensive lesions of the cerebellum typical retrograde changes are observed in the cells of the peri-hypoglossal nuclei in cats 2–3 weeks old. The changes occur throughout all of the nuclei, but are most marked in the caudal third of the nucleus praepositus and the nucleus of Roller. The occurrence of such cell changes is taken to demonstrate the presence of efferent fibres to the cerebellum. Somewhat more than half of these fibres are uncrossed. Small as well as larger cells show retrograde changes after lesions of the cerebellum. This contradicts the assumption that the larger cells are 'motor', the smaller ones 'sensory', and an alternative explanation is offered.

3. After lesions of the brain stem at the pontine level abundant terminal degeneration is seen in the peri-hypoglossal nuclei. Slighter, but convincing degeneration is seen after lesions of the spinal cord. Thus many descending and some ascending fibres terminate in the nuclei.

4. The functional implications of the findings are discussed. The projection on to the cerebellum supports the view that the peri-hypoglossal nuclei are concerned in some way with the motor functions of the tongue. Brief reference is made to findings in the literature concerning the connexions and function of the peri-hypoglossal nuclei.

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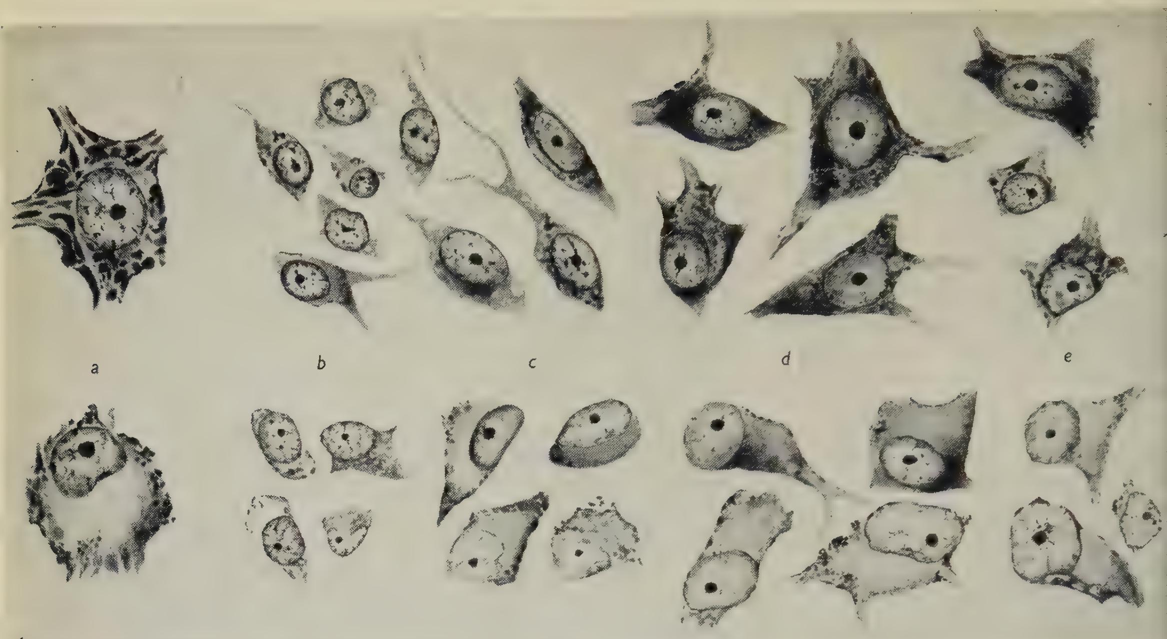

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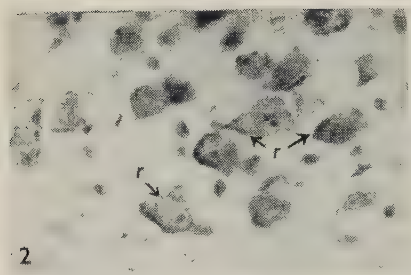

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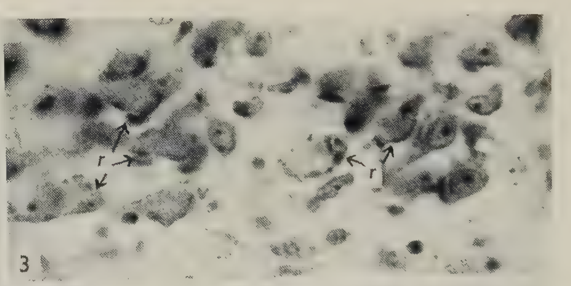

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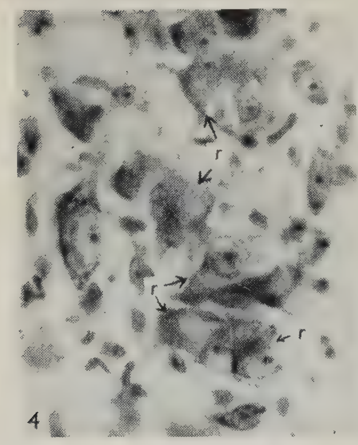

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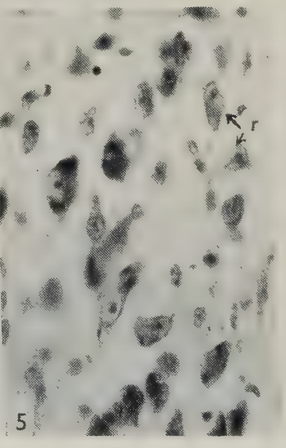

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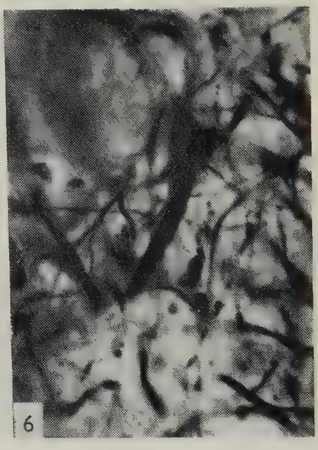

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BRODAL—CONNEXIONS OF PERI-HYPOGLOSSAL NUCLEI

Tagaki, J. (1925*b*). Studien zur vergleichenden Anatomie des Nucleus vestibularis triangularis. II. Vergleichend anatomische Untersuchungen über den Nucleus intercalatus und Nucleus praepositus, das dorsale Längsbündel von Schütz und das Triangularis-Intercalatus-Bündel von Fuse. *Arb. neurol. Inst.* (*Inst. Anat. Physiol. ZentNerv.*) *Univ. Wien*, **27**, 235–282.

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EXPLANATION OF PLATE

Fig. 1. Camera lucida drawings of cells from the hypoglossal and peri-hypoglossal nuclei in the 14 days' old cat. Above, normal cells; below, cells of the various types as they appear when they are fully affected by retrograde (axonal) reaction. *a*, cells from the hypoglossal nucleus; *b*, small cells of the nucleus intercalatus; *c*, medium-sized piriform cells occurring in the rostral part of the nucleus intercalatus and in the nucleus praepositus; *d*, larger multipolar cells from the nucleus praepositus; *e*, cells from the nucleus of Roller. × 600.

Fig. 2. Cells showing retrograde changes (*r*) in the nucleus intercalatus of cat O 93, at a level between drawings 5 and 6 of Text-fig. 2. × 380.

Fig. 3. Cells showing retrograde changes (*r*) in the nucleus praepositus of cat O 93, at the level of drawing 8 in Text-fig. 2. × 380.

Fig. 4. Cells showing retrograde changes (*r*) in the nucleus of Roller of cat O 93. × 380.

Fig. 5. Small cells showing retrograde changes (*r*) in the nucleus intercalatus of cat O 54, contralateral to the side of the lesion. × 380.

Fig. 6. Terminal degeneration in the nucleus praepositus after lesion of brain stem at the pontine level. Glees's method. × 900.

NEUROLOGICAL STUDIES OF THE CARDIAC VENTRICLES OF MAMMALS

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INTRODUCTION

The present histological study of the cardiac ventricles of mammals has three main objects: first, to determine whether or not nerve cells are present; secondly, to observe the types of nerve endings related to the various tissues comprising the ventricles; and thirdly, to investigate the nerve relationships of the atrioventricular part of the cardiac conducting system (the A.V. node and the A.V. bundle and its branches).

Subsequent to the description by Scarpa (1794) and Lee (1849*a*, *b*) of naked-eye 'ganglia' on the epicardial nerves in the ventricles of the cow, horse and man, and the demonstration microscopically by Remak (1838, 1839, 1844) of nerve cells on some of the ventricular nerves in the calf, sheep, pig and man, numerous workers have studied this question. The relevant literature, extensively reviewed by Michailow (1912), Perman (1924), and Stöhr, jun. (1928), reveals that there are wide discrepancies in the observations, even in the results of the studies of the same species of mammal by different investigators. Most observers maintain that cardiac nerve cells are limited to the atria. Only a few describe a widespread distribution of ventricular nerve cells, situated mainly in the epicardium, e.g. Kasem-Beck (1885, 1887) in the dog, calf, sheep and pig; Smirnow (1895, 1905) in the rabbit, hare, squirrel, wolf, dog, cat, calf and man; Valedinsky (1905, 1908, 1910) in the rabbit, dog, calf, pig and man; and Michailow (1912) in the mouse, rat, rabbit, dog, sheep, pig and ape. Smirnow, Valedinsky and Michailow are agreed that the nerve cells are principally multipolar, few being bipolar or unipolar, while Kasem-Beck found many bipolar and unipolar cells as well as multipolars.

Some observers have described organized sensory nerve endings in the ventricular epicardium and endocardium in the form of complicated branchings (Smirnow, 1895), encapsulated arborizations and coils (Michailow, 1912), large diffuse endings (Nettleship, 1936), or tactile corpuscles (Landau, 1950), while others (Fukutake, 1924; Edens, 1924; Woollard, 1926) observed only endings of simple type. Huber & DeWitt (1898) and Fukutake (1924) failed to find any motor end-plates or muscle spindles in relation to the ventricular muscle fibres like those in skeletal muscle, whereas King (1939) described both of these types of endings, and Lavrentiev (1946) reported that Plechkova, working in his laboratory, found proprioceptor endings similar to those in skeletal muscle, as well as true spirals around the myocardial fibres. Woollard (1926), Jones (1927), Boeke (1932) and Tcheng (1951) maintained that nerves penetrate into the substance of the muscle fibres and end near their nuclei.

Several workers have noted that the A.V. bundle is accompanied by nerves, some stressing that these nerves vary considerably in number in different mammals.

Some writers have suggested that it may be the nerves, and not the bundle itself, which conduct the impulse for cardiac contraction from the atria to the ventricles (Agduhr, 1933; Wahlin, 1935; Glomset & Birge, 1945, 1948; Field, 1951).

MATERIALS AND METHODS

The hearts studied comprised representatives of many orders of mammals, as follows, the number of hearts examined from each species being indicated in brackets.

Ornithodelphia: platypus (*Ornithorhynchus*) (1), echidna (*Pro-echidna*) (2).

Didelphia: wallaby (*Macropus robustus*) (2), kangaroo (*M. giganteus*) (1).

Monodelphia: Insectivora: hedgehog (*Erinaceus*) (1), mole (*Talpa*) (2); Rodentia: rat (*Mus*) (3), rabbit (*Oryctolagus*) (4), guinea-pig (*Procavia*) (4); Carnivora: cat (*Felis*) (3), dog (*Canis*) (4); Cetacea: porpoise (*Phocoena*) (2); Artiodactyla: Ruminants—cow (*Bos*) (6), sheep (*Ovis*) (4), red deer (*Cervus*) (2); Non-ruminants—pig (*Sus*) (3); Perissodactyla: horse (*Equus*) (2); Chiroptera: common bat (*Vespertilio*) (2), fruit bat (*Pteropus*) (1); Menotyphla: tree shrew (*Tupaia*) (1); Primates: (*Homo*) (adult, 3; child, 4).

In most cases the hearts were serially sectioned in the transverse plane. In the case of the horse, cow, red deer and pig, and in two of the adult human hearts, only blocks of tissue were sectioned. The following were the principal techniques used: impregnation in bulk by a silver-pyridine method (Blair & Davies, 1935), or staining of individual sections by haematoxylin and eosin, or iron haematoxylin and picro-fuchsin, or by the silver technique of W. Holmes (1947). In addition, in many cases frozen sections of blocks of tissues were treated by the Bielschowsky-Gros technique.

OBSERVATIONS

Nerve cells. Only in Artiodactyla and Cetacea were ventricular nerve cells found to be abundant, lying for the most part in the epicardium, and only in these animals were numerous nerve cells observed in relation to the A.V. bundle and its limbs. No nerve cells were seen in the endocardium or myocardium in any heart examined, with the exception of the cow in which a few were found in the superficial part of the myocardium.

The widespread distribution of epicardial nerve cells in the calf is shown in Pl. 1, figs. 1 and 2, each dot representing a group of cells ranging from about six up to fifty or more. Their positions were determined by graphic reconstruction from serial sections and plotted on photographs of another heart. The cells extend as far as the apex of each ventricle, on both ventral and dorsal surfaces. Many of the cells lie in the course of nerve trunks that are related to the coronary arteries, but some are situated quite apart from the vessels. In the cow and calf epicardial nerves are visible to the naked eye through the intact epicardium and discrete swellings on them, similar to the 'ganglia' described by Scarpa and Lee, could be seen in places where the nerves crossed over or under coronary arteries, though some have no relation to the vessels. Histological examination of several of the swellings showed that, while many are true ganglia, others contain no nerve cells and are produced by a localized thickening of the epineurium. The disposition of endocardial nerve cells in the calf is shown in Pl. 1, figs. 3 and 4; the dots have the same significance and were plotted in the same way as those showing the epicardial cells. They were found to be limited to the A.V. bundle and its limbs (Pl. 2, fig. 9).

In the adult cow and in the sheep, nerve cells were found to be numerous and to be distributed similarly to those in the calf. In the red deer, numerous epicardial nerve cells extend over the proximal three-quarters of both ventricles, but none were found in relation to the A.V. bundle or its limbs. In the pig, epicardial nerve cells extend only for a short distance distal to the coronary sulcus, though in this restricted ventricular territory they are fairly numerous, but none were seen in relation to the A.V. bundle or its limbs. So far as the present work goes, therefore, there appears to be a contrast between the extent of distribution of ventricular nerve cells in Ruminants (cow, calf, sheep and red deer) and Non-ruminants (pig) amongst the Artiodactyla. In the horse, as a representative of the Perissodactyla, by marked contrast with the Artiodactyla, no nerve cells at all were found in the ventricles. In the porpoise, nerve cells were found in great numbers, with a distribution similar to those in the cow, calf and sheep. In the other animals no nerve cells were found in the ventricles, except in the mole, common bat and fruit bat in which a few epicardial cells were seen to extend for a very short distance beyond the coronary sulcus, and in one of the four dogs examined, in which a small group of epicardial cells was seen on the posterior surface of the left ventricle near the coronary sulcus.

In Artiodactyla and Cetacea the nerve cells are frequently arranged in the form of ganglia alongside nerve trunks with nerve fibres passing between the ganglia and the trunks (Pl. 2, fig. 6); in places they form ganglia within the substance of nerve trunks (Pl. 2, fig. 5), and in others a few cells or even single cells occur in the course of nerves. The epicardial nerve cells are mostly bipolar or unipolar (Pl. 2, fig. 10) in type, few being multipolar, whereas those related to the A.V. bundle and its limbs are principally multipolar, few being bipolar or unipolar. In general, the multipolar cells have numerous processes (Pl. 2, fig. 11), most of which enter neighbouring nerve trunks but could not be traced in the sections to their individual destinations; some have, in addition, short processes which end near the cell body in club-like enlargements. In most cases the axon could not be identified, though sometimes a process much thinner than the others was observed which probably represented the axon. The processes of the bipolar and unipolar cells are usually thick. The two processes of the bipolar cells frequently arise from opposite poles of the cell body (Pl. 2, fig. 8); sometimes they arise conjointly from one pole (Pl. 3, fig. 15). Occasionally the single process of a unipolar cell was observed to divide into two some distance from the cell body (Pl. 2, fig. 12), such cells thus resembling pseudo-unipolar cells of spinal and cranial nerve ganglia. This appearance suggested the possibility that the unipolar and bipolar cells might be sensory (afferent) in nature. Frequently the endings of nerve fibres, in the form of small knobs, were seen in relation to the cell bodies and processes of the multipolar cells (Pl. 3, figs. 13, 14), whereas no nerve endings were found in relation to the bipolar and unipolar cells. These observations suggested that the bipolar and unipolar cells might be afferent and the multipolar cells efferent in nature. In general, individual nerve cells are surrounded by a capsule of satellite (neurilemmal) cells (Pl. 2, fig. 12), and the nerve endings related to the multipolar cells lie both outside and inside the capsules.

Nerve endings. The observations on the types of nerve endings in the ventricles were made chiefly on the hearts of the cow, calf and dog. Preparations made by the prolonged osmic acid method of Kiss (1932) showed numerous medullated nerves in the epicardium (Pl. 2, fig. 7) and to a lesser extent in the endocardium and myocardium. Their myelin sheaths vary considerably in thickness and the fibres themselves also vary in diameter. Numerous non-medullated nerves are present in the epicardium and endocardium and many pass from these sites into the myocardium where they form the myocardial plexus. As they approach their terminations all the nerve fibres examined were non-medullated, and it appeared that the medullated nerves lose their myelin sheaths some distance from their terminations. The non-medullated fibres present varicosities along their course, some in the form of spherical knobs, others as elongated fusiform or irregularly cylindrical swellings. In histological sections, these structures, where the nerves surround groups of nerve cells, present a false appearance of knob-like nerve endings, and it is suggested that this may account for the description by other authors of nerve endings in relation to all types of nerve cells. Many neurilemmal cells (Schwann cells) are related to the non-medullated fibres.

The only type of nerve ending found in the epicardium and endocardium was a simple bulb-like enlargement or a gradual thinning leading to an abrupt termination (Pl. 3, fig. 16); no organized endings were seen. No nerve endings resembling the motor endings or muscle spindles of skeletal muscle were observed in relation to the myocardial fibres. It was noted, however, that fine non-medullated nerves ended by forming spirals around individual muscle fibres (Pl. 3, fig. 17). No nerve endings were found in the connective tissue between groups of myocardial fibres. The coronary arteries in the epicardium and myocardium were observed to be accompanied by numerous nerve fibres which course both in the adventitia of the vessels and in the surrounding connective tissue. Simple endings of non-medullated nerves, in the form of very small end-bulbs or a tapering off and sudden ending, were noted in the adventitia of the arteries (Pl. 4, fig. 25), both in the epicardium and myocardium. The capillary vessels were seen to be accompanied by delicate non-medullated nerves and the impression was gained that they comprise the main components of the plexus of nerves in the myocardium, though it was not possible to be certain since the capillary walls were only infrequently and capriciously impregnated and rendered visible by the silver techniques employed. It was clear, however, that the nerves which left the A.V. bundle (*see later*) to pass into the ventricular septum are far too few to contribute materially to the very numerous fibres that enter the septum and the ventricular walls from the epicardium along with branches of the coronary arteries. It was a striking feature that, under the microscope, considerable areas of the ventricular muscle were devoid of nerves, and it is believed that this appearance is not due to any vagaries of the techniques. Fine non-medullated nerves were also seen to pass into the media of the arteries to end as slight enlargements or simply by coming to a sudden end, either between the plain muscle fibres or within them (Pl. 4, figs. 24, 26). It was noted that only in occasional parts of an artery could such nerves be seen passing into the media.

Nerves of the atrioventricular bundle. In Artiodactyla and Cetacea the A.V. bundle was found to be accompanied by numerous nerves (Pl. 4, fig. 23), many of which continued along its two limbs. In the other mammals, although many nerves were seen in relation to the A.V. node, relatively few accompanied the A.V. bundle (Pl. 4, figs. 20, 21), and the limbs of the bundle contained extremely few, the distal part of the limbs containing practically none. In the dog, for instance, no nerves at all were found in the limbs of the bundle in specimens in which the nerves in the A.V. node and bundle and other parts of the heart were very well impregnated with silver (Pl. 4, fig. 22). In Artiodactyla and Cetacea many nerves leave the bundle and its limbs to pass into the ventricular septum, and even in the other animals a number of the nerves related to the bundle were seen to do likewise. In all cases the nerves in the bundle are predominantly non-medullated, few being medullated with thin myelin sheaths. They lie in the connective tissue round the bundle as well as between groups of the muscle fibres comprising the bundle. Nerve endings in the adventitia and media of coronary arteries accompanying the bundle were observed, similar to those described above in the case of the arteries in the myocardium.

Two types of nerve endings were noted in relation to the muscle fibres of the bundle. First, a fine plexus was seen on the surface of the muscle fibres (Pl. 3, fig. 18); it is best developed about the large Purkinje fibres in Artiodactyla and Cetacea, while in the other animals fine nerves lie on the surface of the bundle muscle fibres though a plexus formation is not well developed. No branches from the plexus were observed to penetrate into the substance of the muscle fibres, and it is suggested that these perifibrillar nerves may be sensory in nature. In places the nerves surround individual muscle fibres but often they are related only to groups of fibres, and not all the fibres or groups of fibres are surrounded by such nerves. Secondly, simple nerve endings in the form of terminal knobs were seen on the surface of some of the bundle muscle fibres (Pl. 3, fig. 19), which were epilemmal in position and which it is suggested may be efferent (motor) in nature.

Fine non-medullated nerves were also observed in the endocardium close to the peripheral subendocardial Purkinje fibres, but they were not seen to end in particular relationship to the Purkinje fibres themselves. These nerves have exactly the same features as subendocardial nerves that are not related to Purkinje fibres, they end in the same manner and it is believed that they belong to the general system of subendocardial sensory nerves. Likewise, no nerve endings were seen in relation to the Purkinje fibres that, in Artiodactyla and Cetacea, penetrate into the myocardium from the endocardium.

In all the animals numerous nerves, mostly non-medullated, are related to the A.V. node, and two types of nerve endings were seen in relation to the nodal fibres, similar to those described above related to the muscle fibres of the A.V. bundle. In all cases numerous nerve cells lie in the atrial septum, close to, but not actually in, the substance of the node; they are multipolar (principally), bipolar and unipolar in type, and processes from the cells pass into the node.

DISCUSSION

Among representatives of many orders of mammals, only in Artiodactyla and Cetacea were ventricular epicardial nerve cells found to be numerous and extensive in distribution, a finding contrary to the results of some previous workers who found few or none in the porpoise (Eiger, 1909*a*, *b*) calf (Skworzow, 1874; Perman, 1924), sheep, goat and pig (Perman, 1924). Undoubtedly, there is a variation in the degree to which nerve cells in the other mammals may extend beyond the coronary sulcus, as witnessed by the finding of a few ventricular epicardial cells in one of the dogs examined, and it is believed that such variation accounts for other workers finding some ventricular cells in mammals in which none were found in the present work (Schklarewski, 1872; J. Dogiel, 1877, 1895, 1914; Vignal, 1881; Aronson, 1886; Weinreich, 1888; Jacques, 1894; A. S. Dogiel, 1899; Noc, 1899; Fahr, 1909, 1910; Müller, 1911). It is submitted that this widespread distribution of ventricular nerve cells in Artiodactyla and Cetacea is a true morphological difference between these two orders and the others, since no functional adaptation is apparent. The absence of ventricular nerve cells in the horse as a representative of the Perissodactyla, contrary to the condition in Artiodactyla, lends support to the modern tendency, based on other morphological differences, to discontinue the term 'Ungulates' as including both these groups and to raise each to equivalent ordinal rank. Simpson (1945) noted the difficulty of assigning a place to the Cetacea in a *scala naturae* of mammals. Boyden & Gemeroy (1950) by precipitin tests showed that the serum proteins of Cetacea resemble those of Artiodactyla more than those of any other existing order of mammals (all except the Lagomorpha being included in their tests). The present finding regarding ventricular nerve cells is a further indication of the close relationship between these two orders. Amongst the Artiodactyla, the Ruminants show a much more extensive distribution of ventricular nerve cells than the Non-ruminants, and in this respect they resemble the Cetacea, a feature that supports other morphological evidence of affinity between these two groups.

Numerous nerve cells were observed in relation to the A.V. bundle only in the porpoise, calf, cow and sheep, and this agrees with the findings of Tawara (1906) in the calf, Wilson (1909), Meiklejohn (1913, 1914) and A. H. Holmes (1921) in the calf and sheep, Scaglia (1927) and Blair & Davies (1935) in the cow, Glomset & Glomset (1940) in the sheep, and Engel (1910) and Perman (1924) in 'Artiodactyla'. Their presence was denied by Morison (1912) in the sheep, and by Truex & Copenhaver (1947) in the calf and sheep. Wilson (1909) and Glomset & Glomset (1940) described them in the pig, Lawrentjew & Gurwitsch-Lasowskaja (1930) in the rat, and Stotler & McMahon (1947) in the dog. Akkeringa (1949) described numerous 'interstitial cells', which he claimed are nerve cells, in relation to the peripheral subendocardial Purkinje fibres in the cow and horse, but no undoubted nerve cells, comparable in size and structure with those described above, were found in these sites by the present workers.

Woollard (1926) and Lawrentjew (1929), on the basis of degeneration studies following excision of sympathetic or vagal nerves, and Nonidez (1939), who noted that sympathetic cells stain less deeply than parasympathetic with his modification of Cajal's chloral-hydrate-silver technique, concluded that all cardiac nerve cells are efferent parasympathetic in nature. The present writers, on purely morphological grounds, suggest that the multipolar ventricular nerve cells may be efferent, and the bipolar and unipolar cells afferent in function. Similar types of nerve cells were noted in the atria, and the same suggestion is made for them. It has not been possible to make out any connexions between the bipolar or unipolar cells and the multipolars, nor to trace the processes of individual cells to their destination, so that the nerve endings described could not be related to the particular types of nerve cells.

The simple nerve endings observed in the epicardium and endocardium are most probably sensory in function. Lawrentjew (1927) and Nettleship (1936) claimed that the nerve endings in the endocardium degenerate after section of the vagus, and Lavrentiev (1946) by section of various nerves (vagal, sympathetic, spinal) concluded that the sensory nerves in the endocardium are vagal and those in the epicardium sympathetic. Weddell & Zander (1950) found that simple nerve endings in the cornea could be damaged during histological preparation so that they become distorted and made to resemble complicated endings. Such as explanation may account for some workers (Smirnow, 1895; Michailow, 1912; Nettleship, 1936; Landau, 1950) describing complicated endings in the epicardium and endocardium.

Tcheng (1951) described what he considered to be sensory nerve endings, in the form of simple knobs, between the *atrial* muscle fibres in the dog, but in the present work, no nerve endings were found in the connective tissue between the ventricular myocardial fibres. It is, therefore, suggested that the simple endings observed in the adventitia of the coronary arteries, which are undoubtedly sensory in nature, may be the ones concerned in the production of cardiac pain. If the mechanism of production of cardiac pain involves ischaemia of the myocardium with the accumulation of pain-producing metabolites that stimulate pain nerve endings (Lewis, 1947), it would appear that such metabolites might diffuse into the adventitia of the arteries and affect these nerve endings. In this connexion, it is noteworthy that, in dogs, traction on a ligature around a coronary artery produces evidence of referred pain (Sutton & Lueth, 1930), whereas puncturing the heart wall appears to be painless (Singer, 1926; Sutton & Lueth, 1930).

Stöhr jun. (1932) could not find nerves in the media of arteries. Woollard (1926) and Blair & Davies (1935) found nerve endings in the media of the coronary arteries, while Boeke (1932) noted that in arteries a single nerve ending could only now and then be found between the muscle cells, and remarked that such an appearance is due to vagaries of the techniques employed. The present writers also noted that the nerve endings in the media of the coronary arteries were only to be found in occasional parts of an artery, and submit that this may be the actual method of innervation of the vessels.

Nonidez (1939, 1943) claimed that his silver technique enabled sympathetic nerves to be distinguished from parasympathetic, and maintained that the atria are supplied by the parasympathetic and the ventricles by the sympathetic. No such distinction could be made in the present study. Plechkova (1936) and Lavrentiev (1946) maintained that in their terminal parts one and the same neurilemmal sheath contains a sympathetic and a parasympathetic axis cylinder, and that the antagonistic action of these two parts of the autonomic nervous system on the heart muscle is thus brought about peripherally, i.e. on the same muscle fibre. Such an arrangement was not observed in the present work.

It is suggested that the spiral nerve endings around the myocardial fibres described above may be of the nature of sensory stretch receptors. If this be so, the fact that they are not extremely numerous may be attributed to the syncytial nature of the myocardium. Stretch receptors in the ventricular myocardium have been postulated by previous workers on the basis of a reflex fall of blood pressure and slowing of the heart rate following upon a rise of pressure in the left ventricle

(Jarisch & Richter, 1939; Richter & Amann, 1940), raising the pressure in the ascending aorta (Daly & Verney, 1926), or injection of veratrum alkaloids into various cardiac chambers or into branches of the coronary arteries (Dawes, 1947).

Many workers have noted that the A.V. bundle is accompanied by nerves, which are mainly non-medullated; e.g. Tawara (1906), Retzer (1908), DeWitt (1909), Wilson (1909), Engel (1910), Morison (1912), Meiklejohn (1913, 1914), A. H. Holmes (1921), Fukutake (1924), Woollard (1926), Scaglia (1927), Wolhynsky (1928), Lawrentjew & Gurwitsch-Lasowskaja (1930), Blair & Davies (1935), Vitali (1937), Glomset & Glomset (1940), Nonidez (1943), Glomset & Birge (1945, 1948), Truex & Copenhaver (1947), Stotler & McMahon (1947), Akkeringa (1949) and Field (1951). Some of the above have noted that the number of nerves varies considerably in different mammals, but all who have studied the Artiodactyla agree that in them these nerves are numerous. Only few nerves were found in the bundle in man by DeWitt, Meiklejohn, A. H. Holmes and Blair & Davies, and in the dog and cat by DeWitt and Meiklejohn, while no nerves at all were seen in man by Fahr (1910) or in the dog by Tcheng (1951). Field (1951), on the other hand, remarked that the richness of the nervous component of the bundle in the dog is striking and is of special interest as this animal has been so widely used experimentally in work on the conduction of the cardiac impulse. The plexus of nerves on the surface of the bundle muscle fibres observed in the present work has also been noted by Retzer, Wilson, Meiklejohn, Woollard, Blair & Davies, Vitali and Akkeringa. It is suggested that these nerves are sensory in function, a belief expressed also by Wilson, Vitali and Blair & Davies, while Meiklejohn and Akkeringa maintained that branches from the plexus penetrate the muscle fibres, the latter author referring to the places of penetration as the 'innervation points' of the muscle fibres. It is suggested that the simple knob-like nerve endings on the surface of the bundle muscle fibres are efferent (motor) in function. Truex & Copenhaver found only simple nerve endings in relation to the bundle muscle fibres and maintained that they lie both on the surface of, and inside the muscle fibres, while Blair & Davies maintained that these endings were hypolemmal in position. Scaglia found nodose endings of nerves on the surface of, and inside, the bundle muscle fibres, as well as endings in the form of various configurations, some encapsulated, in the connective tissue between the muscle fibres. Nonidez described nerve endings in the form of rings, club-shaped enlargements and reticulated swellings on the surface of, and between, the muscle fibres, and maintained that the bundle and the proximal parts of its limbs are supplied by the parasympathetic, though he could not decide whether they are also supplied by the sympathetic since these nerves are not stained by his technique. Woollard concluded that it is highly probable that the bundle is supplied by both sympathetic and parasympathetic nerves.

Several workers, reviewed by Davies & Francis (1946), on the basis of cutting, ligurating or compressing the A.V. bundle and thereby producing dissociation between atrial and ventricular contraction, concluded that the bundle conducts the impulse for cardiac contraction, though some writers have stressed that in experimental lesions involving the bundle (Cohn & Trendelenburg, 1910; Glomset & Birge, 1948) the nerves accompanying the bundle are of necessity involved. Obviously the experiments are not 'crucial' in determining which of the two components of the bundle, muscle or nerves, conducts the impulse. Agduhr & Strenström (1928) and Agduhr (1933) noted that, in mice, cod-liver oil produced focal areas of myocardial degeneration that may involve the A.V. bundle without producing heart-block, and, as apparently undamaged nerves in the bundle were seen in one case, Agduhr suggested that it may be the nerves that are the true conducting elements. Wahlin (1935) also observed sclerotic patches involving the bundle without producing heart-block but only a prolongation of the normal *P–R* interval in the electrocardiogram. Field (1951) was impressed by the presence of nerves in the bundle in several mammals examined by him and quotes the work of Agduhr and Wahlin as casting serious doubts on the myogenic theory of cardiac conduction. Glomset and his colleagues (Glomset & Glomset, 1940; Glomset & Birge, 1945, 1948) could find no anatomical evidence, in the form of an A.V. node and bundle, in the dog, rhesus monkey and man, to support the myogenic theory of cardiac conduction, postulating that the cardiac musculature is under the control of the intrinsic nerve cells and nerves. In the case of the dog (Pl. 4, fig. 22) and man, the present writers disagree with these observations, and Baird & Robb (1950) have described the A.V. node and the bundle and its limbs in the dog as exactly comparable with these structures in Ungulates.

In the present work, the nerves accompanying the A.V. bundle and its limbs were found to be relatively few in number, except in Artiodactyla and Cetacea. Many of them were noted to leave the bundle in the early part of its course to pass into the myocardium of the ventricular septum, an observation also made by Scaglia (1927) and Field (1951), but it appears to the present writers that these nerves are far too few to contribute materially to the plexus of nerves in the septum, the latter being mainly derived from the epicardial nerves. This observation, coupled with the failure to find any motor nerve endings in the myocardium, leads to doubt as to whether the nerves accompanying the bundle can be concerned with the conduction of the impulse from atria to ventricles. Further, if these nerves did convey the impulse to the septal musculature, it might be expected that the upper part of the septum would be activated first, since the earliest nerves to leave the bundle enter this part of the septum, and that the process of activation would extend downwards through the septum. The extensive investigations in dogs by Sodi-Pallares, Rodrigues, Chait & Zuckermann (1951), however, show that activation of the ventricular septum is *from below upwards*, and that the times of arrival of the waves of activation at different points on the right and left surfaces of the septum are in conformity with the distribution of the branches of the two limbs of the bundle. In the present work it was found that the bundle in the dog contains relatively few nerves and that many of these leave the bundle to pass into the upper part of the septum, while the limbs of the bundle contain no nerves at all. It is submitted that these observations, considered in conjunction with those of Sodi-Pallares *et al.*, are strongly in favour of the conduction of the impulse from atria to ventricles by the bundle and its limbs.

Like Nonidez (1943), Truex & Copenhaver (1947), Stotler & McMahon (1947) and Tcheng (1951), the present writers found no nerve endings in relation to the peripheral Purkinje fibres, and, as no nerve endings were seen in relation to the ventricular myocardium that could be interpreted as motor endings, they agree with the opinion of Stotler & McMahon that the relation of the muscle elements of the A.V. bundle and its branches to the ventricular myocardium is best explained by the orthodox conception of the myogenic propagation of the cardiac impulse from atria to ventricles. Collateral evidence, derived from a comparative study of electrocardiograms, that the rate of propagation of the cardiac impulse throughout the ventricles in a number of mammals (and birds) can be correlated with the degree of histological differentiation of the A.V. bundle and its branches, will be presented in another paper by two of the present authors (Davies & Francis).

SUMMARY

1. Only in Cetacea and Artiodactyla, among representatives of a large number of Orders of mammals, were nerve cells found to be numerous and extensive in distribution in the ventricles. This resemblance between these two Orders is adduced as further support for their close systematic relationship.

2. Unipolar, bipolar and multipolar cells were found in all the hearts. From the morphological appearances and the presence or absence of nerve endings in relation to the cells, it is suggested that the multipolar cells may be efferent and the unipolar and bipolar cells afferent in function.

3. Only simple nerve endings were found related to the various tissues comprising the ventricles. It is suggested that the spiral nerve endings around ventricular myocardial fibres are sensory stretch receptors and that the simple nerve endings in the adventitia of the coronary arteries may be concerned with the cardiac pain mechanism.

4. Nerves related to the atrioventricular bundle are described and reasons are given for believing that they are not concerned with conducting the impulse for cardiac contraction from atria to ventricles.

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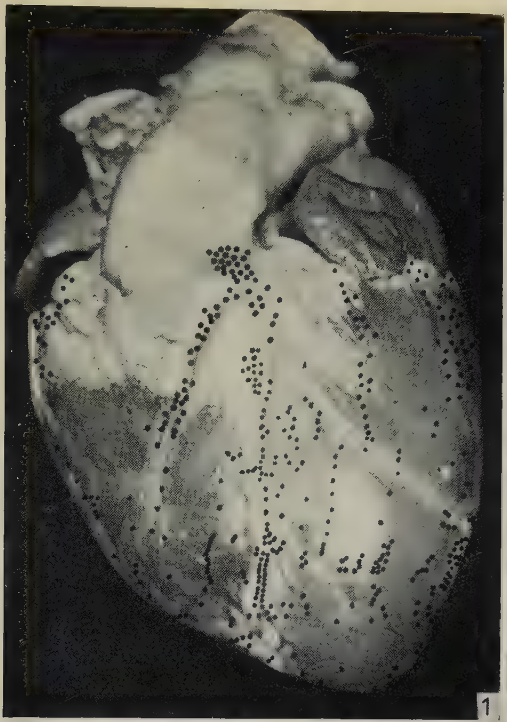
EXPLANATION OF PLATES

PLATE 1

Fig. 1. Ventral surface of heart of calf showing disposition of epicardial nerve cells. × $\frac{3}{8}$ natural size.

Fig. 2. Dorsal surface of heart of calf showing disposition of epicardial nerve cells. × $\frac{3}{8}$ natural size.

Fig. 3. Interior of right atrium and ventricle of heart of calf. The dots indicating the position of subendocardial nerve cells obviously follow the path of the A.V. node, the A.V. bundle and the proximal part of its right limb. × $\frac{3}{8}$ natural size.



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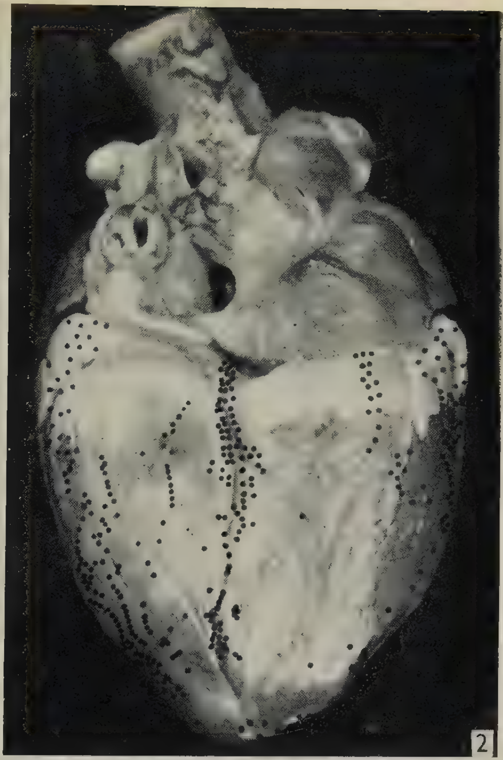

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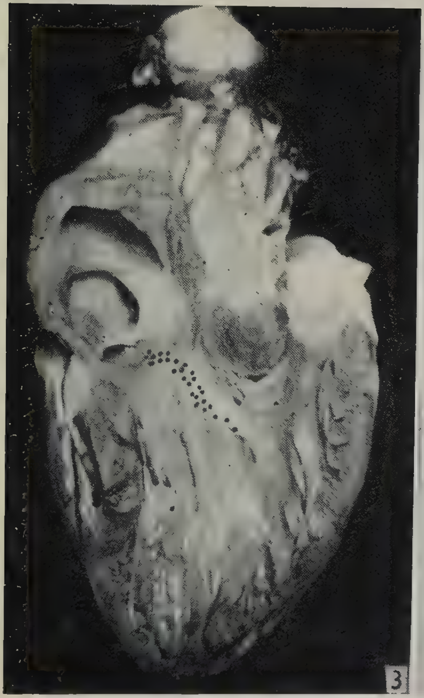

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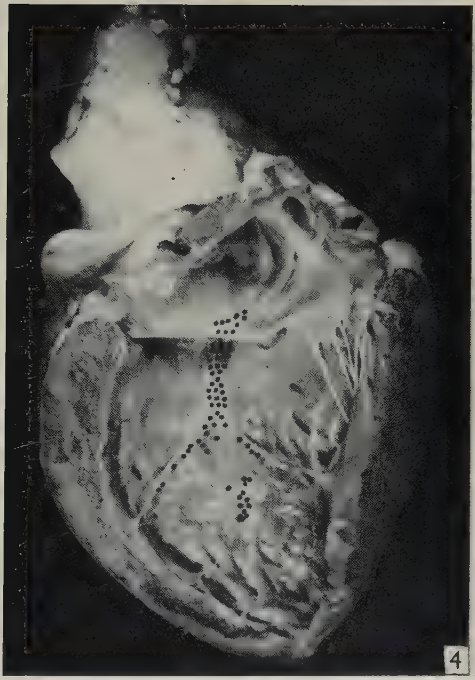

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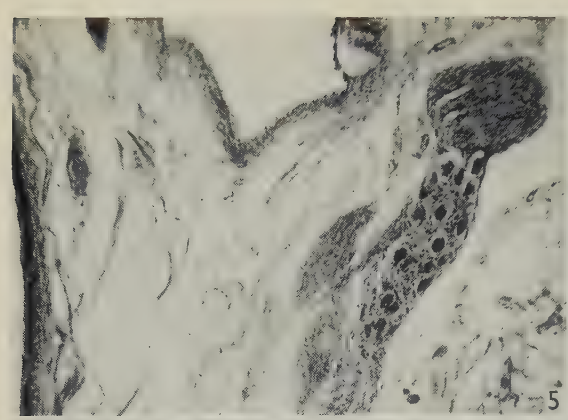
DAVIES, FRANCIS AND KING—NEUROLOGICAL STUDIES OF THE CARDIAC VENTRICLES OF MAMMALS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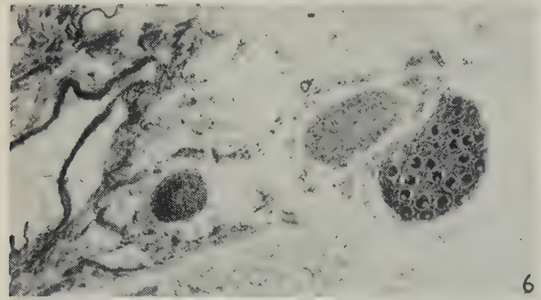
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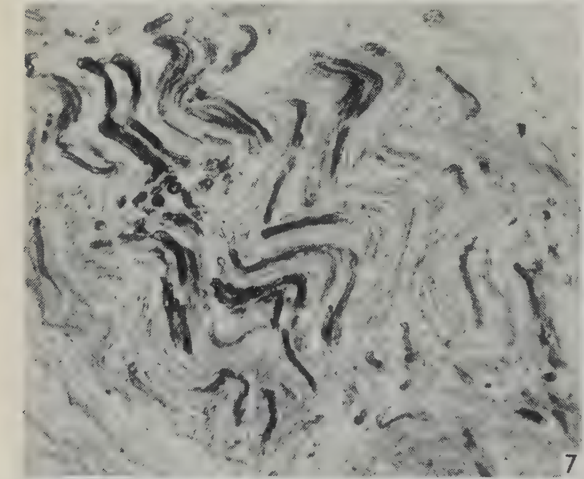
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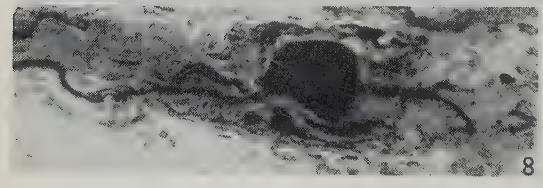
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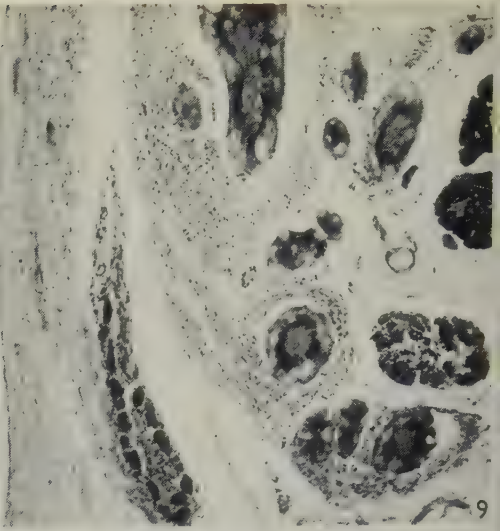
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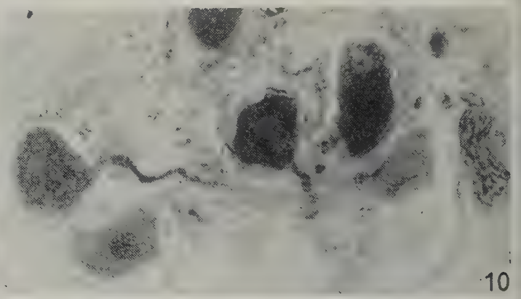
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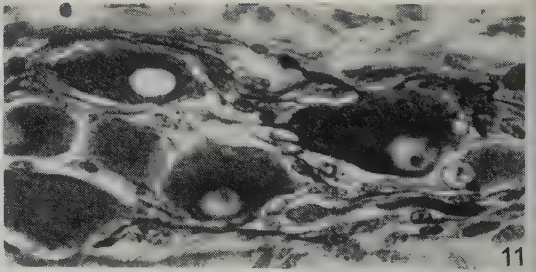
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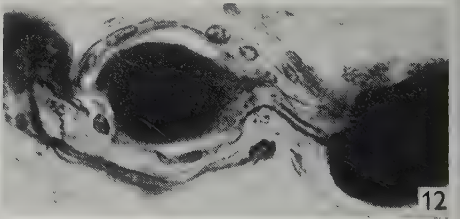
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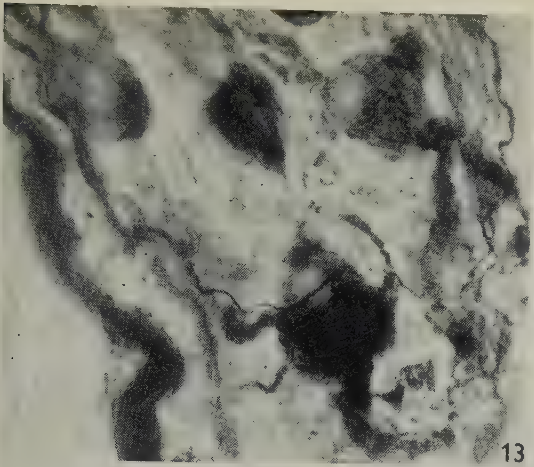
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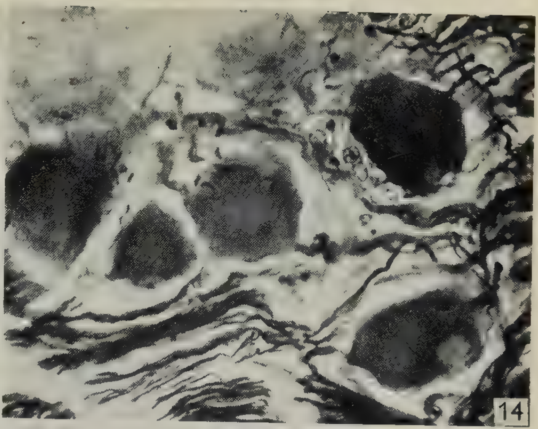
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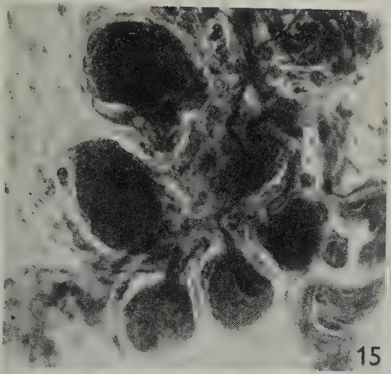
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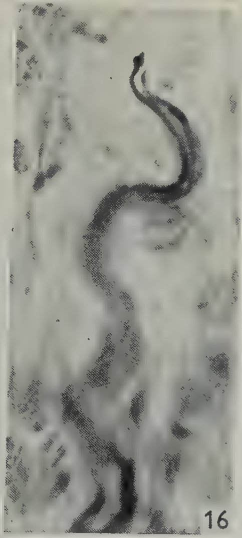
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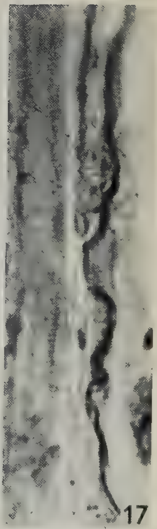
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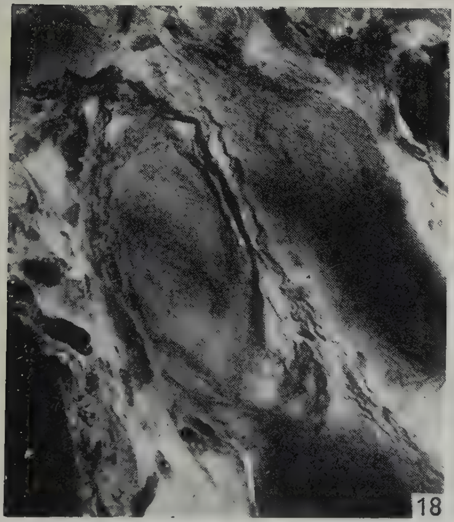
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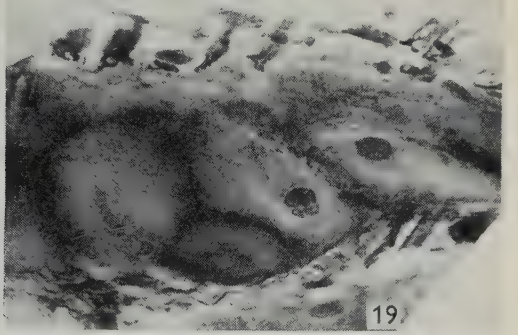
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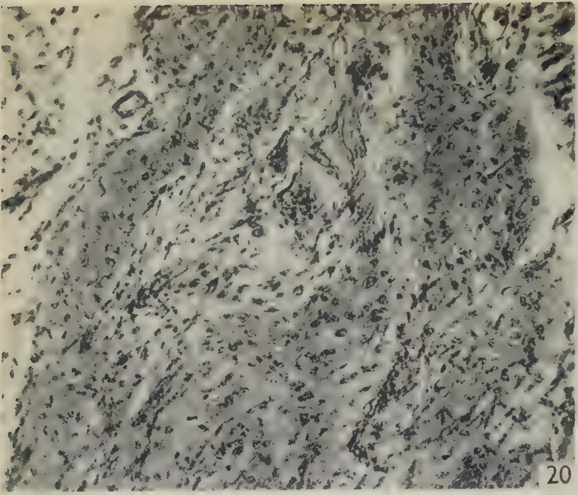
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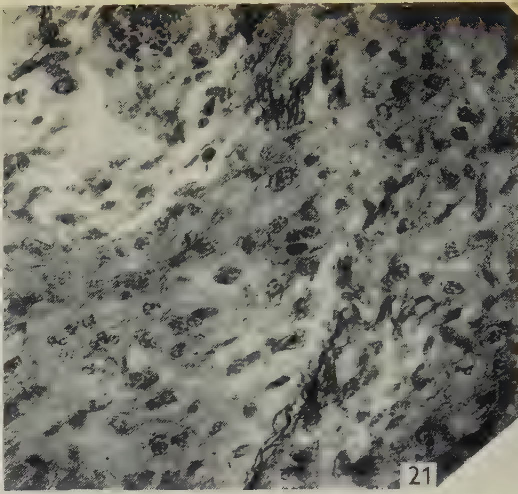
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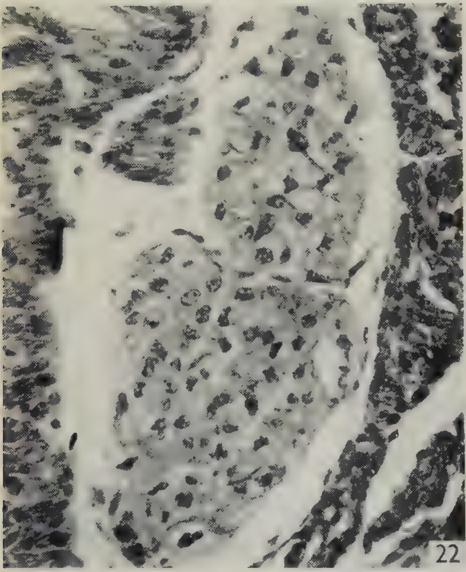
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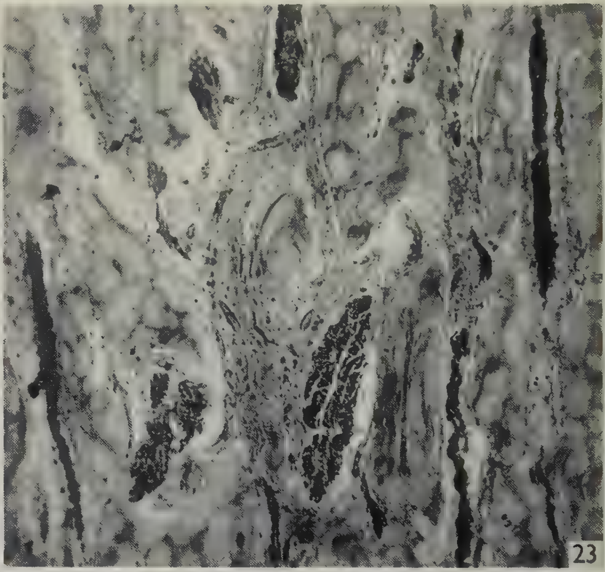
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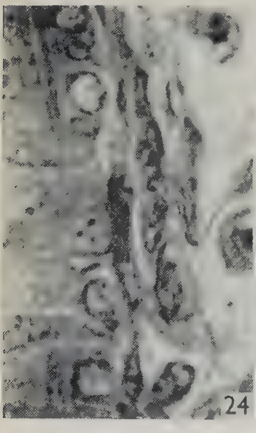
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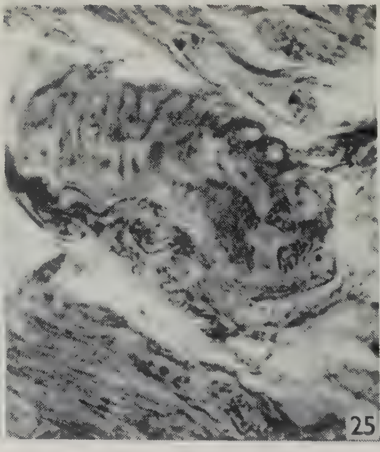
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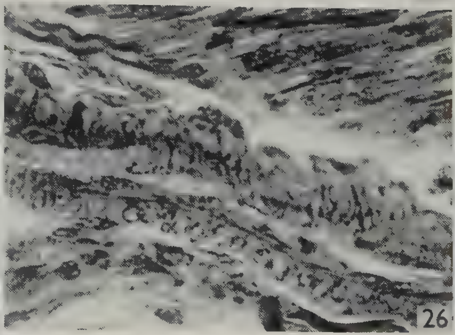
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DAVIES, FRANCIS AND KING—Neurological studies of the cardiac ventricles of mammals

Fig. 4. Interior of left atrium and ventricle of heart of calf. The dots indicating the position of subendocardial nerve cells obviously follow the path of the left limb of the A.V. bundle and some of its major branches. × ⅔ natural size.

Plate 2

Fig. 5. Nerve cells in course of nerve trunk in epicardium of right ventricle of calf. Silver-pyridine. × 100.

Fig. 6. Nerve cells forming ganglion alongside nerve trunk in epicardium of left ventricle of calf. Silver-pyridine. × 62.

Fig. 7. Medullated and non-medullated nerve fibres in epicardium of right ventricle of cow. Osmic acid. × 260.

Fig. 8. Bipolar nerve cell in epicardium of left ventricle of cow. Bielschowsky-Gros. × 350.

Fig. 9. Nerve cells alongside left limb of A.V. bundle of calf. Silver-pyridine. × 112.

Fig. 10. Unipolar nerve cells in epicardium of right ventricle of calf. Bielschowsky-Gros. × 400.

Fig. 11. Multipolar nerve cells in A.V. bundle of cow. Silver-pyridine. × 480.

Fig. 12. Unipolar cells in epicardium of right ventricle of cow. Note that the central nerve cell is surrounded by satellite cells and that its process passing to the right divides into two a short distance from the cell body. Bielschowsky-Gros. × 435.

Plate 3

Fig. 13. Nerve cells in epicardium of left ventricle of cow. Note nerve fibre (derived from another cell) winding around the left and right processes of the multipolar cell in the lower part of the field. Bielschowsky-Gros. × 440.

Fig. 14. Nerve endings in the form of small knobs in relation to nerve cells and their processes. Epicardium of left ventricle of cow. Holmes silver. × 410.

Fig. 15. Nerve cells in epicardium of left ventricle of calf. Note two processes arising conjointly from upper surface of the lowermost cell. Bielschowsky-Gros. × 390.

Fig. 16. Simple nerve endings in epicardium of right ventricle of calf. Holmes silver. × 900.

Fig. 17. Nerve fibre forming spiral around individual muscle fibre in left ventricle of calf. Bielschowsky-Gros. × 690.

Fig. 18. Plexus of nerve fibres on surface of Purkinje fibres in A.V. bundle of cow. Silver-pyridine. × 420.

Fig. 19. Simple nerve endings on surface of Purkinje fibres in A.V. bundle of cow. Silver-pyridine. × 410.

Plate 4

Fig. 20. A.V. bundle of dog. Note relative paucity of nerves. Holmes Silver. × 164.

Fig. 21. Part of previous figure under higher magnification. × 300.

Fig. 22. Upper part of right limb of A.V. bundle of dog to show complete absence of nerve fibres. Note differentiation of muscle fibres of the right limb from those of the surrounding myocardium. Holmes silver. × 340.

Fig. 23. A.V. bundle of cow. Note profusion of nerves. Holmes silver. × 92.

Fig. 24. Note nerve ending in right part of figure among muscle cells of the media of a small coronary artery. Dog. Holmes silver. × 1220.

Fig. 25. Note nerve endings in adventitia of a coronary artery. Dog. Holmes silver. × 920.

Fig. 26. Note nerves ending among muscle cells of the media of a coronary artery. Dog. Holmes silver. × 620.

STUDIES OF THE RENAL BLOOD SUPPLY IN FOETAL AND NEONATAL ANIMALS

By ALISON M. FERGUSSON

Department of Physiology, St Bartholomew's Hospital Medical College, London

It is now well established that infant renal physiology differs from adult, but so far anatomical explanations for the differences have been largely conjectural.

Dean & McCance (1949) compared the renal responses of infants and adults to hypertonic solutions of chloride and urea, and concluded that infants must have immature glomerular function. They suggested that this might be due either to the high visceral epithelium covering the glomerular tuft, or to some glomeruli being outside the main renal blood-flow in foetal and neonatal life.

West, Smith & Chasis (1948), on the basis of mannitol and PAH clearances, reported a very high filtration fraction in infancy, and they thought it might be due to post-glomerular vascular channels, the blood in which did not reach the tubules. Smith (1951) has since modified this viewpoint, concluding that the more likely explanation is deficient development of proximal tubular tissue, leading to incomplete tubular extraction. He points out, however, that if extraction ratios in infancy should prove to be normal, re-interpretation would be necessary.

Trueta, Barclay, Daniel, Franklin & Prichard (1947) found, on examining kidneys from young human foetuses, that the juxtamedullary glomeruli and the medulla were packed with red cells, in contrast with the relative bloodlessness of the rest of the cortex. They suggested that the juxtamedullary glomeruli and their associated circulatory system might have a particular significance at this stage of development.

In view of these speculations on the pathway of the renal circulation in early life, it seemed of interest to inject foetal and neonatal animals *in vivo* with indian ink, and study the distribution of dye in the kidney by histological methods. Results show the vascularization to be normal, and indicate that the functional differences are partly due to glomerular immaturity.

MATERIAL AND METHODS

The neonatal animals used were twenty-six rabbits, aged 0–26 days, four guinea-pigs and three kittens. The foetuses used were seven rabbit and two guinea-pig litters, totalling sixteen subjects.

Neonatal animals. The guinea-pigs were anaesthetized with pentobarbital sodium (nembutal veterinary solution), injected intraperitoneally in the dosage of 0·44 ml. per kg. body weight. The other animals were all anaesthetized with ether, with the exception of two rabbits, anaesthetized with urethane, injected intraperitoneally in the dosage of 2 g. per kg. body weight (25 % solution).

The abdomen was opened and the abdominal aorta was gently cleared. No attempt was made to ligate the mesenteric artery or to pass a thread round the renal pedicle preparatory to ligation, as such interference was found to produce paling of the kidneys, particularly if the anaesthetic was at all light. From 0·4 to 2·0 ml. of indian ink at body temperature was injected retrogradely into the abdominal aorta with a hypodermic needle, usually of 19- or 20-gauge. The pressure of the injection was such as to keep the column of dye just above the origin of the renal arteries, so that it was carried into the kidneys at approximately the animal's own blood pressure. The injection was continued until the renal venous blood was seen to darken, and the renal pedicle was then clamped. The kidneys were removed and fixed for preference in Susa, but occasionally in 10 % formol saline. They were then embedded in paraffin, cut at 6–10 μ, and counterstained with haematoxylin and eosin. Several makes of indian ink were used, the most satisfactory available being Pelican drawing ink.

Foetal animals. The pregnant rabbits were anaesthetized with pentobarbital sodium in the dosage of 0·44 ml. per kg. body weight, and ether. The abdomen was opened and the uterus incised over one foetus. The umbilical vessels could sometimes be seen clearly through the membranes, but if these were opaque they were incised locally, and indian ink at body temperature was injected into the umbilical vein. According to Popoff (1938), the rabbit has one umbilical vein and one vitelline vein, and Schultze (1897) found that there was considerable anastomosis between the placental and vitelline vessels in the rabbit, so that injection through the umbilical vein should not obstruct the circulation completely. The injection was made with a 19- or 20-gauge hypodermic needle, and 1–2 ml. of ink were introduced. At the end of the injection the umbilical cord was clamped, and the foetus and placenta removed. Dissection of the kidneys was sometimes carried out immediately, sometimes after fixation of the whole foetus in Susa. The foetal age of the rabbits ranged from 19 to 30 days, the gestation period being 30–31 days. After fixation of the kidneys in Susa or 10 % formol saline, they were treated in the same way as the neonatal kidneys.

One pregnant guinea-pig was anaesthetized with pentobarbital sodium intraperitoneally, in the same dosage as the neonatal animals, the other with 25 % urethane solution injected intraperitoneally in the dosage of 2 g. per kg. body weight. The uterus was opened and the injection carried out in the same way as with the rabbits, and the foetal kidneys were dissected out and fixed in Susa.

RESULTS

Neonatal rabbits. On introducing the ink into the aorta, the renal cortex usually flushed black immediately. On section, ink was found in almost every glomerulus, and there were traces of ink in the characteristic S-shaped developing glomeruli of the nephrogenic zone. In addition, the peritubular capillaries were outlined with ink. Usually the cortex became injected before the medulla, the vasa recta not being filled with ink unless the injection was continued after the dye first appeared in the renal vein.

In seven out of twenty-six subjects, injection of the cortical glomeruli was incomplete; in some only the juxtamedullary glomeruli were injected, in others all the glomeruli supplied by one interlobular artery were injected, while those adjacent were uninjected, except for the juxtamedullary ones. In these cases the kidney was usually noticed to be paler than normal before the injection, and on introducing the dye, the surface of the kidney blackened patchily or in streaks, instead of flushing black immediately. In several cases the glomeruli were better injected around the hilum than on the lateral surface of the kidney, and even when the glomeruli were not injected, the peritubular capillaries in the area contained ink. The rabbit's kidney at birth still has a well-marked nephrogenic zone, and this was found to persist for about 14 days. The juxtamedullary glomeruli at birth (Pl. 1, fig. 1) were not unlike those of the adult, having well-developed capillary loops, and only traces of the high visceral epithelium which is a characteristic of all mammalian kidneys, including the human, in foetal life (Klein, Burdon-Sanderson, Foster & Brunton, 1873). The glomeruli of the outer part of the cortex (Pl. 1, fig. 2) were much more primitive in form, being considerably smaller, and consisting of simple capillary loops surrounding a central core of connective tissue. They were covered by a complete layer of cubical or columnar epithelium, which separated the capillary from the capsular space.

Neonatal kittens. Two newborn subjects and one 3-week-old kitten showed complete injection of the glomeruli. Like the rabbit, the kitten's kidney was found to have a nephrogenic zone at birth, and the juxtamedullary glomeruli were better developed than the outer ones. (Pl. 1, figs. 3, 4.)

Neonatal guinea-pigs. Two animals showed almost complete injection of the glomeruli, one being newborn and the other aged 2 days. (Pl. 2, fig. 5.) In the other two animals, both newborn, the surface of the kidneys was seen to become injected patchily, and many of the outer glomeruli in each case remained free of ink.

The guinea-pig's kidneys were found to be more mature at birth than those of the other animals studied, having the full number of glomeruli and a well-marked cortex corticis; that is, a layer of convoluted tubules between the outermost glomeruli and the capsule, as described by Peter (1928) in human kidneys. There was little difference in size between the outer and inner glomeruli, though the outer ones still showed traces of high visceral epithelium. The outer glomerular tufts were considerably better formed than those in the rabbit or cat.

Foetal rabbits. Completeness of injection in the foetal rabbits varied, but injection of the glomeruli was noticeably better in the first foetus to be injected from each doe. In some experiments several foetuses had been used for other purposes before an injection was made into those remaining, and in these cases the glomeruli were poorly filled, presumably owing to deterioration of the condition of the mother and so of the circulatory conditions in the foetus.

In the doe of 19 days' gestation the second foetus was injected, attempt at injection of the first having been unsuccessful. The sections made from this animal showed both mesonephros and metanephros. The mesonephros had conspicuously large glomeruli which were well injected. The metanephros had a layer of well-formed juxtamedullary glomeruli, which contained ink, and two or three layers of developing glomeruli, some of which contained traces of ink, and almost all of which contained red blood corpuscles. The peritubular capillaries were quite well injected, though some of these may have filled from the venous side. The medulla consisted of groups of large collecting tubules embedded in primitive connective tissue, and contained some relatively large arterial and venous branches in which ink was present.

In a doe of 20 days' gestation the second foetus was injected, after the first foetus had been removed rapidly without attempt at injection. This animal was fixed whole in Susa, and the kidneys dissected out next day. The mesonephros (Pl. 2, fig. 6) contained large, very well-injected glomeruli, and most of the metanephric glomeruli (Pl. 2, fig. 7) also contained ink, including the outer ones, which were only just beginning to form. The histological picture of the kidney was not noticeably different from that of the foetus of 19 days' gestation.

In a doe of 21 days' gestation the first and second foetuses were injected. The first foetus was better injected than the second; in both cases the juxtamedullary glomeruli contained ink, but in the second foetus there was very little to be seen in the outer glomeruli. The section of the second foetus showed a small part of the mesonephros, the glomeruli of which contained traces of ink.

By 23 days' gestation there were three layers of formed glomeruli in the foetus studied, and traces of ink were seen in almost every glomerulus. The number of convoluted tubules was greater, and the medulla showed well-developed vasa recta, together with large collecting tubules, but the loops of Henle were still inconspicuous.

In the doe of 25 days' gestation the foetus to be injected was the last of four, and the mother had previously been given 0·3 mg. of adrenaline intravenously, which was found to have produced macroscopic paling of the kidneys of the third foetus to be removed, compared with those of the first foetus, removed before the adrenaline injection. In this case the glomeruli were poorly injected, traces of ink being confined to the juxtamedullary ones, whereas the peritubular capillaries were well outlined. The vasa recta were congested with ink and blood.

By 28 days the appearance of the foetal kidney was little different from that of the newborn rabbit, and the glomeruli of the foetus studied, which was the first to be removed, were well injected.

In the doe of 30 days' gestation the second foetus was injected, and showed ink in almost all glomeruli, including some which were only just forming.

Foetal guinea-pigs. The foetuses of two pregnant guinea-pigs were injected via the umbilical vein. The exact gestation age was not known, but both were near term, and each doe had four foetuses, the average crown-rump length of one group being 12 cm. and of the other 11·5 cm. The crown-rump length of newborn guinea-pigs was found to be 13–14 cm. In every foetus all the glomeruli were already formed, although the high visceral epithelium of the outer glomeruli was rather more prominent than at birth. In each case the first foetus to be injected showed ink in almost all glomeruli, but in the other three only the inner glomeruli were injected.

DISCUSSION

These results suggest that the normal pathway of the intrarenal circulation is the same in foetal and neonatal animals as it is in adults. The glomeruli appear to be included in the circulation at an early stage of their development, and no difference could be found in the pattern of the peritubular capillaries or in the circulation through the medulla. In most cases the cortex was better injected than the medulla, which indicates that the main pathway of the circulation is through the glomeruli and cortical tubules.

Of the seven neonatal rabbits in which injection of the cortical glomeruli was incomplete, some had been difficult to anaesthetize and had reacted to incision of the abdominal wall; in one case a thread had been passed round the renal pedicle, preparatory to ligature, and this may have stimulated the renal nerves mechanically; in another case the aorta had been exceptionally difficult to clear. In two cases urethane had been used, and may have had a depressant effect.

Trueta *et al.* (1947) have shown that paling of the renal cortex, associated with progressive shutting off of the outer layers of glomeruli, may be produced by adrenaline, stimulation of somatic afferent nerves, and stimulation of the renal nerves. Franklin and Miles (unpublished observation, 1950) found that a mechanism for paling the renal cortex is present in rabbits long before birth, paling having been observed on administration of adrenaline to the mother, and on crushing the foetal brain after removing the foetus from the uterus. Anoxia was found to pale the kidneys of newborn rabbits, as it does in adults (Franklin, McGee & Ullmann, 1949).

The incomplete injections observed in these cases were almost certainly due to the conditions of the experiment, which in one way or another set in motion the mechanism for shutting off the outer glomeruli from the circulation, and the nineteen rabbits in which injection of virtually all glomeruli occurred, probably represent the normal state.

Of the species studied, the rabbit and cat are born blind, and in a more immature state than the human infant. This is reflected in the kidneys, which show a prominent nephrogenic zone, whereas in the full-term human infant all the glomeruli are formed (Potter & Thierstein, 1943). Glomerular development in man has, however, only just reached a stage of completion at birth, in that Potter & Thierstein found, in general, that infants weighing less than 2·1 kg. still showed a zone of developing glomeruli. The guinea-pig is born in a considerably more mature state, being able to feed itself on the first day of life, and the number of renal glomeruli seems to be complete some time before birth. Tubular development also seems to be in advance of the human species, for a cortex corticis is present at birth, whereas in the infant it is not fully developed for some months (Peter, 1928).

Since species born both less and more mature than the human being have essentially the same intrarenal vascular pattern at birth as in adult life, it seems probable that there is no fundamental difference between the circulatory pathways of the infant's kidney and those of the adult.

Trueta *et al.* (1947) suggested that the juxtamedullary glomeruli had an especial significance in early life, but these results suggest that they are important mainly in that they are the first to be formed, and that as soon as other layers of glomeruli appear, the circulatory pathway extends to them also. This is supported by a section from a 20-week-old human foetal kidney, in which glomeruli from all layers of the cortex were packed with red cells.

Study of the individual glomeruli, however, suggests a possible reason for the low rate of glomerular filtration in the immature kidney. In the rabbit and cat at birth, the outer glomeruli have a complete layer of cubical or columnar visceral epithelium and the capillary loops are relatively undeveloped, and appear to be completely separated from the capsular space by the high epithelium (Pl. 1, figs. 2, 3). Not only must the quantity of blood flowing through the glomerulus in a given time be considerably less than in the adult, owing to the poorly developed vascular tuft, but filtration must occur across a thick epithelium, instead of the thin membrane of the adult. It is interesting that developing mammalian glomeruli seem to be not unlike Marshall's (1934) description of those of adult birds and reptiles, which have poor glomerular filtration. Baxter & Yoffey (1948) commented on the simplicity of the capillary loops in the glomeruli of the newborn rat, and they found that trypan blue given subcutaneously to these animals was only apparent in the convoluted tubules of the inner part of the cortex; it is possible that in this species, and maybe in the newborn rabbit and kitten, very little or no filtration occurs through the outer glomeruli.

In the earliest foetal rabbits studied, the juxtamedullary glomeruli were considerably better developed than are the outer cortical glomeruli at birth, and from their histological appearance it would seem that filtration could occur through these glomeruli at an early stage of foetal life. According to Gersh (1937), the foetal rabbit of 19 days' gestation has a functioning mesonephros, but the metanephric glomeruli do not excrete sodium ferrocyanide, although they do not appear histologically different from those of a rabbit of 21 days' gestation, which do excrete this substance. It is of interest that the 19-day foetal rabbit kidney in this series showed injected glomeruli, suggesting that it is not a different pathway of the renal circulation, but some other factor, which determines the onset of filtration.

In the guinea-pig at birth the vascular tuft of the glomeruli is well developed (Pl. 2, fig. 5), although there are still traces of high epithelium to be seen in the outer glomeruli. Renal function in the newborn guinea-pig seems to be more advanced than in other species studied. Dicker & Heller (1951) found that the glomerular filtration rate, measured with inulin, was at comparable levels in newborn and adult guinea-pigs at high rates of urine flow, though the glomerular filtration rate varied with the urine flow in the newborn animals, unlike the adults. The guinea-pig appears to be in advance of the human species in this respect, for the inulin clearances of newborn infants have repeatedly been found to be considerably below adult levels, compared on a basis of surface area, even at high urine flows (Barnett, 1940; Barnett, Perley & McGinnis, 1942; McCance & Young, 1941; Dean & McCance, 1947).

Histologically, the development of the glomeruli seems to be a gradual process in the species studied, which is in keeping with the findings of Bevis & Schofield (1951) in the human being, rather than with the view of Gruenwald & Popper (1940), who postulated a sudden expansion of the capillary loops and bursting of the epithelium at birth. There seems to be no dramatic change in the structure of the kidney, or in the pathways of the renal circulation, to explain Williamson & Hiatt's (1947) findings of a greatly increased phenol red excretion by rabbits immediately after birth, and it would seem more probable that such changes in function are due, first, to the removal of the placenta, which is the preferential organ of excretion in foetal life, and secondly, to a rise in blood pressure at birth, such as was observed in the rabbit by Hamilton, Woodbury & Woods (1937).

SUMMARY

1. The distribution in the kidney of indian ink, injected intravascularly *in vivo*, was studied in various foetal and neonatal mammals by histological methods.

2. The findings suggest that the pathway of the intrarenal circulation is essentially the same in late foetal and neonatal life as it is in the adult animal.

3. It is suggested that the low glomerular filtration rate in infancy is due to the relatively undeveloped state of the vascular tuft in the outer glomeruli, and to the high epithelium lining the visceral layer of Bowman's capsule.

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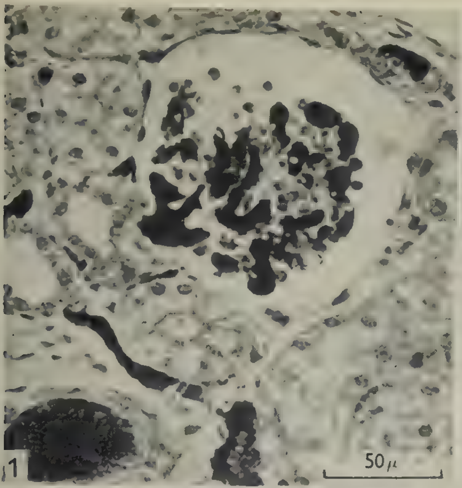
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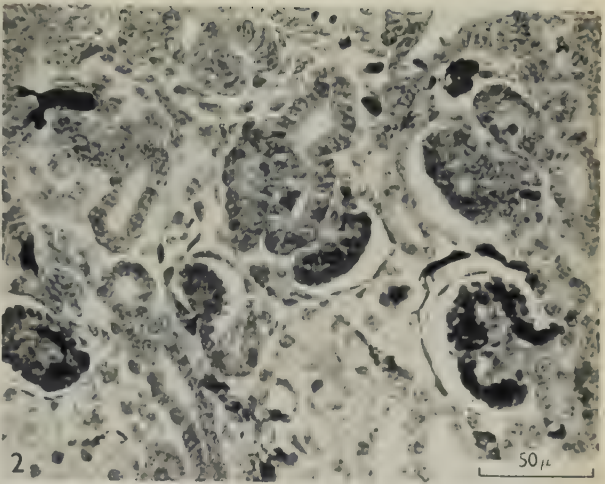
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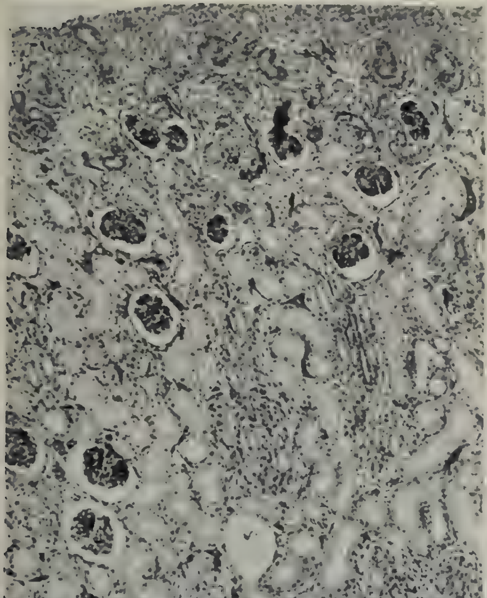
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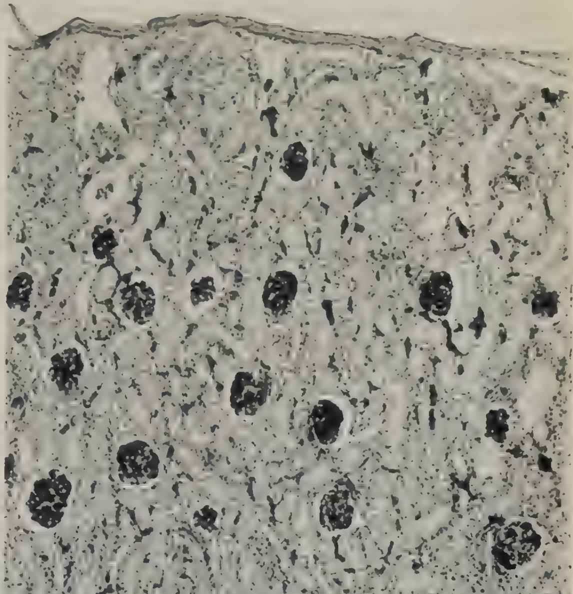
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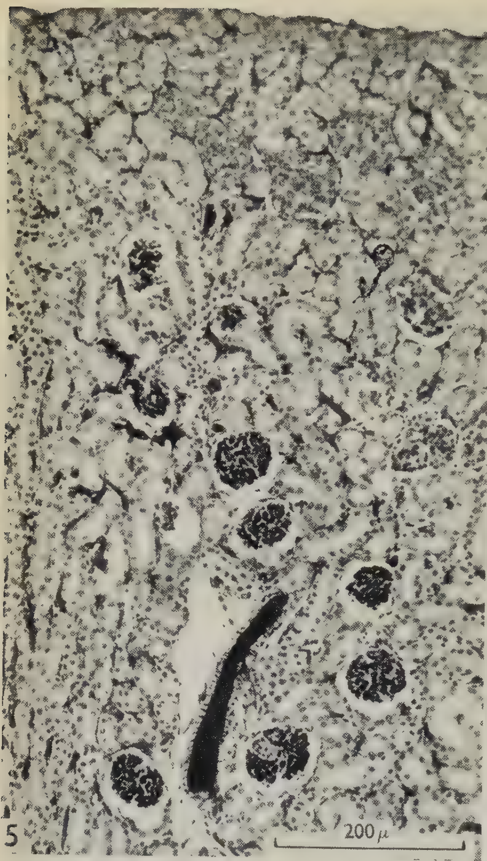
FERGUSSON — STUDIES OF THE RENAL BLOOD SUPPLY IN FOETAL AND NEONATAL ANIMALS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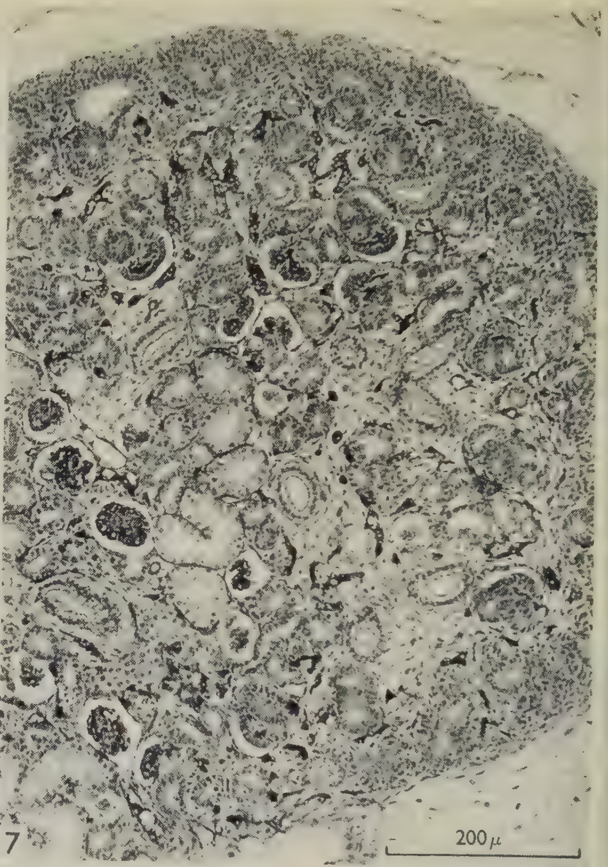
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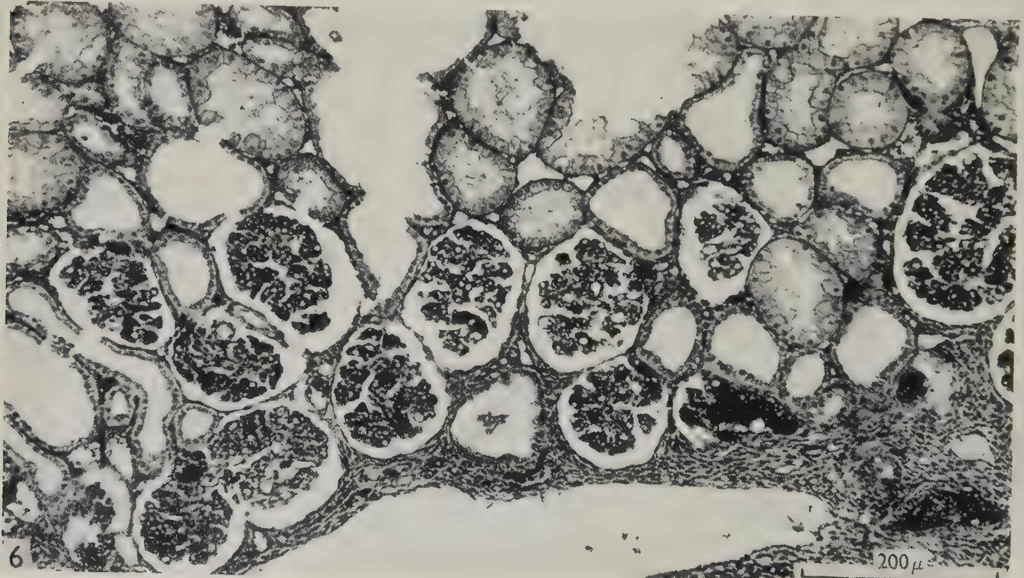
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FERGUSSON—Studies of the renal blood supply in foetal and neonatal animals

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Newborn rabbit's kidney. Two juxtamedullary glomeruli injected with indian ink.

Fig. 2. Same kidney. Outer cortical glomeruli, showing high visceral epithelium and simple capillary loops.

Fig. 3. Newborn kitten's kidney. Well-marked nephrogenic zone. Outer glomeruli smaller and simpler than juxtamedullary ones.

Fig. 4. Kitten aged 3 weeks. All glomeruli better developed. Well-marked cortex corticis.

PLATE 2

Fig. 5. Newborn guinea-pig's kidney. Glomeruli at all levels are injected, but a group of glomeruli on the right have remained uninjected.

Fig. 6. Foetal rabbit of 20 days' gestation. Mesonephros. Large, well-injected glomeruli.

Fig. 7. Same animal. Metanephros. Section taken to one side of hilum. Wide nephrogenic zone. Ink in glomeruli at all stages of development.

THE CONDUCTING (CONNECTING) SYSTEM OF THE CROCODILIAN HEART

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INTRODUCTION

Most of the observations by a large number of workers, reviewed by Davies & Francis (1946), are in favour of a myogenic rather than a neurogenic initiation and conduction of the impulse for contraction in the vertebrate heart, and the present histological study of the crocodilian heart is in consequence concerned with the investigation of the muscular connexions between its several chambers. In studying the problem of the phylogeny of the cardiac conducting system of mammals and birds the crocodilian heart assumes special importance since there occurs in it, for the first time in the vertebrate series, a complete ventricular septum. Thus such questions as the following arise. Does the presence of a complete ventricular septum modify the arrangement of the muscular atrioventricular connexions that exist in lower vertebrates so that the connexion is now situated solely or mainly in the ventricular septum, as in mammals and birds, or do the peripheral atrioventricular connexions, that precede any septal connexions in phylogeny, still form the main or only muscular connexion between atria and ventricles which could form a pathway for the passage of the impulse for cardiac contraction? Further, are the atrioventricular muscular connexions constituted by ordinary cardiac muscle or by specialized muscle fibres comparable in structure with those in mammals and birds?

In spite of the importance of these considerations, surprisingly few investigations have been made of the connexions between the sinus venosus and atria and between the atria and ventricles in the crocodilian heart. The older anatomical literature, now classic, was concerned purely with naked-eye morphology and not with matters that relate to the cardiac rhythm; it is well summarized by Röse (1890) and Hoffmann (1890). The most comprehensive anatomical description of the crocodilian heart (alligator and crocodile) is that of Greil (1903), who gave a detailed account of the morphology of the heart and its several chambers and of its valves and septa. Greil, however, did not clearly distinguish between striated cardiac muscle and smooth muscle or between structures derived from cardiac and from arterial sources. The work of Keith & Flack (1907), in which the sinu-atrial node of the mammalian heart was first described, following closely as it did on the observations on the atrioventricular node and bundle by Tawara (1906), was largely preoccupied with the phylogeny of these structures, and they claimed that they are remnants of more extensive tissues of similar nature that they stated were present at the junctions of the various cardiac chambers in lower vertebrates. Their colleague, Mackenzie (1913), studied the crocodilian heart (alligator and crocodile). He found that the sinus venosus established continuity with the right atrium by means of 'nodal tissue' situated on the left side of the sinu-atrial orifice. So far as the connexions of the atria with the ventricles are concerned, he maintained that continuity of the left atrium with the left ventricle is interrupted by fibrous tissue at the bases of the membranous atrioventricular valves, and that on the right side, in addition to occasional muscular continuity between the right atrium and right ventricle in the atrioventricular funnel, the right atrium is connected to the ventricular septum by means of 'nodal tissue'. Mackenzie believed that the connexions between the chambers of the crocodilian heart foreshadow the mammalian condition. Mangold (1914*a*, *b*) summarized the current views on the conducting system of the vertebrate heart but made only passing reference to the crocodilian heart, while Goodrich (1919), reviewing the morphological relations of the ventricular septum in the main reptilian orders (including Crocodilia), confined his attention to gross anatomy and made no reference to any connexions that might act as a conducting pathway or to any histological features. Swett (1923) described the muscular connexions between the several cardiac chambers of *Alligator mississippiensis* and was unable to confirm Mackenzie's description of 'nodal tissue' at the junctional sites. He found that the sinus muscle is continuous with the musculature of the right atrium all around the sinu-atrial orifice, that the musculatures of the atrial septum and of both atria are continuous with that of the dorsal part of the ventricular septum, and that peripheral connexions exist between the atrial and ventricular musculatures at the dorsal and right-dorsal quadrants of the right atrioventricular orifice and to a lesser extent at the dorsal and left-dorsal segments of the left atrioventricular orifice, these peripheral atrioventricular connexions involving the atrioventricular funnel. He also stated that 'experiments which have been carried out in Doctor Laurens's laboratory on the hearts of young alligators have substantiated the above anatomical description of the course of the auriculo-ventricular connection'. Lowman & Laurens (1924) took kymographic records from the right atrium and the apex of the ventricles before and after making scissors cuts in various segments of the atrioventricular junction and concluded that the most important single part of the atrioventricular connexion for coordinating atrioventricular rhythm is the right-lateral, other important parts being the left-lateral and left-ventrolateral, while the dorsal and right-dorsolateral parts are the least important. They also maintained that this differentiation agrees with the structure of the connexions as determined by Swett, but it appears to the present authors that these conclusions are at considerable variance with the anatomical muscular connexions determined by Swett and with those found in the present work.

MATERIAL AND METHODS

Histological study was made of five hearts of *A. mississippiensis* (juvenile), which had been fixed in 10 % formalin and sectioned serially at 10 μ in each of the three conventional planes—transverse, frontal and sagittal, and of one heart of *Crocodilus niloticus* (juvenile), similarly fixed and sectioned serially at 10 μ in the transverse plane. The stains employed were iron haematoxylin and picrofuchsin, or haematoxylin and eosin, or Mallory's connective tissue stain, or Bodian's activated protargol. One alligator heart was impregnated in bulk by a silver pyridine method (Blair & Davies, 1935) and serially sectioned at 10 μ in the frontal plane. The grosser anatomical and topographical details, worked out from the sections, were supplemented by dissections of fresh hearts of *Alligator mississippiensis* and *Crocodilus niloticus*. Graphical reconstructions were made of the junctional regions to assist interpretation.

OBSERVATIONS

The bulk of the histological observations were carried out on the alligator hearts, but no significant differences were observed in the single heart of the crocodile studied.

The sinus venosus. The sinus venosus, which forms a separate cardiac chamber, lies dorsal to the right atrium and has the form of an elongated pyramid, the short base of which lies cranially and the apex caudally (Pl. 1, fig. 2; Pl. 2, figs. 6, 7). The two precaval veins enter the lateral angles of its base, the postcaval and the two hepatic veins enter its apex, while the coronary vein enters its ventral surface near the apex. The muscle fibres constituting its dorsal wall run mainly transversely, though there is some criss-crossing of the fibres. Towards the ventral aspect, i.e. towards the junction of the sinus with the right atrium, the muscle fibres change their direction and become mainly longitudinal. At the actual site of junction of the sinus with the right atrium there is not a compact ring of muscle as in the Salamander (Davies & Francis, 1941); rather does the musculature of the sinus communicate with that of the right atrium through a broad meshwork of fibres, largely circular in direction (i.e. surrounding the orifice), but with a considerable admixture of connective tissue. A short distance proximal to the sinu-atrial junction, however, there is a thickened ring of circular muscle fibres in the sinus wall (Pl. 2, fig. 7). Two large muscular valves, right and left (Pl. 2, figs. 6, 7), guard the opening of the sinus into the right atrium. The muscular walls of both the sinus and the right atrium contribute to their formation, and there is quite free continuity between these two muscular components of the valves all around the sinu-atrial orifice. At the left margin of the sinu-atrial opening the wall of the right atrium is thickened (Pl. 2, fig. 7), the basket-work of atrial muscle fibres here being infiltrated with connective tissue. In the same area there are a few large nerve ganglia and many nerve fibres. It appears to the present writers that this arrangement of muscle, nerve cells and nerves is the structure described by Mackenzie (1913) in the crocodile as the sinu-atrial node, but it should be noted that the above arrangements involve the atrial and not the sinus muscle, and further that no histological differences could be made out between the muscle fibres here and those elsewhere in the atrial walls. The presence of nerve cells and the considerable admixture of connective tissue in this site do not warrant the identification of this site as 'nodal tissue'.

The atria. The right atrium lies rather to the ventral aspect, and the left atrium towards the dorsal aspect of the heart (Pl. 1, fig. 1). Each has an auricle (appendix) of considerable size. The atrial walls, as well as the atrial septum, consist of a basket-work of muscle fibres, with prominent muscular trabeculae which form a loose spongework projecting for a short distance into the atrial cavities.

The atrioventricular junctions. The atria are slightly invaginated like a funnel into the base of the ventricles (Pl. 2, fig. 9), the connective tissue of the epicardium following this invagination and to a large extent effecting a separation of the musculatures of the atria and ventricles except towards the caudal extremities of the funnel, where in places the atrial muscular component of the funnel is continuous with the ventricular component (Pl. 2, fig. 10). The connective tissue invaginated into the funnel is more marked in the left chamber than in the right. On the right side some straggling muscle fibres penetrate the connective tissue at the base of the funnel to effect union between the atrial and ventricular muscle, but such muscular continuity is not present all round the atrioventricular orifice. On the ventral aspect of the atrioventricular orifice, where the bases of the large arteries are situated, there is no muscular continuity between the right atrium and right ventricle in the funnel, either at its base or in its caudal part. Thus atrioventricular continuity of the right side is limited to the dorsal, right-dorsal and right segments of the atrioventricular orifice. On the left side there is no penetration of muscle fibres through the connective tissue at the base of the funnel, and continuity of the left atrial and ventricular muscles at the caudal part of the funnel is limited to the dorsal and left-dorsal segments of the atrioventricular orifice, beneath the base of the marginal cusp of the atrioventricular valve. At the base of the funnel the muscle fibres of both atria take a circular direction and form an atrioventricular 'ring' (Pl. 2, figs. 9, 10), though the 'ring' is incomplete ventrally where the large arteries take their origin from the heart, and thus has a rather C-shaped form. It is, nevertheless, interposed between the general musculature of the atria and the atrioventricular funnel, and it is suggested that the time taken for the passage of the cardiac impulse through this 'ring' probably accounts for the interval between atrial and ventricular contraction, which was observed in the exposed heart of the living crocodile.

On the right side the atrioventricular funnel is much more extensive, and passes more deeply into the ventricle, than on the left side (Pl. 2, fig. 9). The caudal border of the right part of the funnel is to some extent free from attachment to the interior of the right ventricle, but it is doubtful whether it is free enough to allow the funnel to act as a valvular cusp (marginal cusp; Pl. 2, fig. 11) in the act of closure of the right atrioventricular orifice during ventricular systole in the manner suggested by Greil (1903). It would appear, judging solely from the morphological arrangements, that the only effective cusp at the right atrioventricular orifice is the septal cusp. It is fibrous in structure, and its basal attachment extends for about two-thirds of the circumference of the atrioventricular orifice. Its caudal border is free, except at its ventral part which is attached by a stout muscular band to the interior of the ventricular wall (Pl. 2, fig. 11). The contraction of this muscular band during ventricular systole would have the effect of shortening the free border of the cusp, thereby adjusting it to the contracting atrioventricular orifice and at the same time preventing evagination of the cusp into the right atrium. In the avian heart there is no septal cusp at the right atrioventricular orifice, and the closure of this opening during ventricular systole is effected solely by the muscular valve that lies in relation to the free wall of the ventricle. This avian valve is homologous with the right part of the atrioventricular funnel in the crocodilian heart described above, but the valvular function of the latter would appear to be incipient, rather than actually attained.

The left atrioventricular orifice is guarded by a pair of large membranous semilunar valves, one septal and one marginal (Pl. 1, fig. 5). The septal cusps of the right and left atrioventricular valves are attached to the corresponding surfaces of the pars membranacea septi, but the attachment of the septal cusp of the left valve is situated some distance cranial to that of the right valve, the part of the pars membranacea septi intervening between the two valves representing morphologically the proximal portion of the septal cusp of the right valve. No direct continuity of the musculatures of the atrial and ventricular septa was found, these being separated by the pars membranacea septi. The marginal cusp of the left atrioventricular valve is as well developed as the septal cusp and its base is attached to the caudal border of the atrioventricular funnel. The caudal margins of both cusps are free, there being no chordae tendineae.

So far as continuity of the musculature of the atria with that of the ventricular septum is concerned, the conditions differ in the case of the two atria. There is no continuity whatever between the left atrial musculature and the ventricular septum, a stout mass of connective tissue intervening between the dorsal wall of the left atrium and the dorsal part of the base of the ventricular septum (Pl. 1, figs. 4, 5). By contrast, the dorsal wall of the right atrium establishes an obvious muscular connexion with the base of the ventricular septum; indeed, the most substantial muscular connexion between the right atrium and the ventricles occurs at this site, the dorsal wall of the atrium being continuous with both the free wall of the right ventricle and the ventricular septum (Pl. 1, figs. 4, 5).

The above description of the peripheral muscular connexions between the atria and ventricles agrees with that of Swett so far as the actual limits of the connexions are concerned, but Swett claimed that the finer structure of the connecting musculature resembles that of the atrial muscle, and that its distribution to, and mingling with, the ventricular fibres are easily observed, inasmuch as the striations of the ventricular fibres are much wider and take the stain (iron haematoxylin) more deeply. With the staining techniques described above, or using phase-contrast microscopy of unstained sections, this has not been substantiated. No difference could be found in appearance or in size (breadth) between the junctional fibres and the ventricular or atrial muscle. In all parts of the heart there is some slight variation in the appearance of the striations in the fibres seen in any one histological section, and these differences are attributed to the actual state of the fibres at the time of fixation rather than to a constant histological difference.

The structure labelled 'bulbus' by Swett is really the bases of the aortic arches bound together in a common sheath, and consists solely of fibrous tissue and smooth muscle, with some cartilage, at the bases of the vessels where they join the ventricles. Greil (1903) also regarded these structures as bulbus tissue. There is, however, a remnant of the bulbus in the form of a muscular band, composed of cardiac muscle, which spirals around the base of the pulmonary arch, and the pulmonary arch alone (Pl. 2, fig. 8). Naturally it is continuous with the musculature of the right ventricle. It was recognized by Mackenzie but Swett appears to have overlooked it.

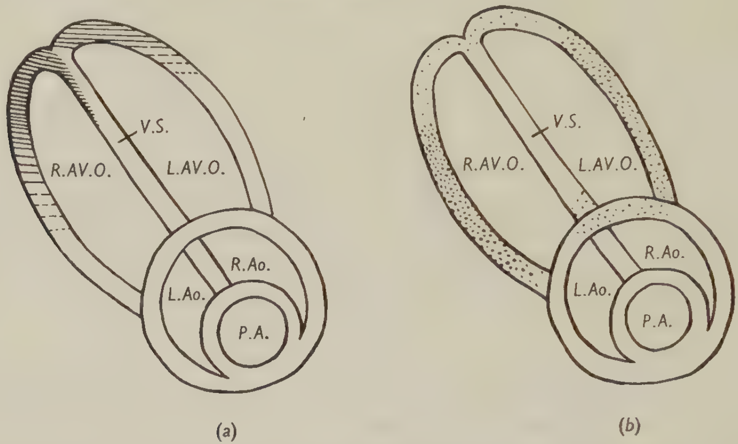
From the ventral surface of the sinus venosus the epicardium is reflected to the dorsal surface of the base of the right ventricle, near the dorsal interventricular sulcus, in the form of the dorsal ligament or sinuventricular fold (Pl. 1, figs. 2, 3). As Swett observed, it consists of fibrous tissue and contains the coronary vein, a small artery, and some nerve fibres and nerve cells. It does not contain any muscle and, therefore, does not constitute a muscular connexion between the cardiac chambers. It is comparable in structure and position with the dorsal ligament described by Laurens (1913*a*, *b*) in the lizard and tortoise and shown experimentally by this author, contrary to the findings of Dogiel (1907) and Imchanitzky (1909), to have no significance for the co-ordination of the heart's action.

Microscopic structure of the cardiac musculature. So far as the finer structure of the muscle in the crocodilian heart is concerned, no differences were found between the characters of the fibres of the several chambers. Measurements of numerous fibres taken at random from each chamber in the heart of the alligator show that the breadth of the fibres ranges from 7·5 to 10μ, the great majority being 8·3μ broad. These figures hold uniformly for each of the chambers and for the connecting muscle fibres at the sinu-atrial and atrioventricular junctions. The coarseness of the striations also varies somewhat, but the differences between the fibres of any one chamber are quite as great as can be found between those of different parts of the heart. The whole myocardium of the heart, therefore, presents a very uniform histological picture, and no histological specialization has been found in any part of the heart of the alligator or crocodile comparable with nodal tissue or Purkinje fibres in the hearts of mammals and birds.

DISCUSSION

The results of the present study of the crocodilian heart indicate that the muscular connexions between the sinus venosus and the right atrium, and between the atria and ventricles consist of ordinary heart muscle, and that the cardiac muscle of the several chambers and at the junctional sites presents a uniform histology throughout. It is suggested that the pauses between the contractions of the sinus and atrium on the one hand, and between the systole of the atria and that of the ventricles on the other, such as were observed in the heart of the living crocodile, are due to the ring-like arrangement of the muscle fibres situated proximal to each junctional site, operating on a similar principle to that described in the heart of the salamander (Davies & Francis, 1941). At the sinu-atrial junction the ring is complete, but at the atrioventricular junction the ring is interrupted ventrally by the fibrous tissue at the bases of the large arteries so that the delaying musculature here forms a 'C' rather than a complete ring.

The advent of the ventricular septum has resulted in the establishment of a substantial connexion between its musculature and that of the right atrial wall. This muscular connexion occupies a different morphological site from that of the atrioventricular node and bundle of the mammal and bird, and it is believed that it is not the true homologue of these latter structures. Davies & Francis (1941) have suggested that the sinu-atrial node, the atrioventricular node and the atrioventricular bundle and its branches are neomorphic developments in mammals and birds and it is now maintained that they are not foreshadowed in the crocodilian heart. It may be affirmed with certainty that in the crocodilian heart the sinu-atrial and atrioventricular muscular connexions show no histological specialization comparable with those in mammals and birds. Further, the peripheral atrioventricular connexions, which involve the atrioventricular funnel and which precede phylogenetically the septal connexions, persist to some extent in the crocodilian heart, though unlike those in lower vertebrates they are interrupted ventrally by fibrous tissue. In mammals and birds these peripheral connexions are normally entirely interrupted by fibrous tissue, the sole atrioventricular muscular connexions in these animals being by means of the specialized atrioventricular bundle and its branches. It has already been reported (Davies, Francis & King, 1951) that there are a number of differences between the electrocardiographic tracings obtained from *C. niloticus* and those from man, which may be accounted for by the absence of specialized conducting muscle in the crocodilian heart.



Text-fig. 1. Diagrams of the atrioventricular junctional region of the heart of the alligator. (*a*) The muscular connexions between the atria and ventricles described in the present paper are indicated by cross-hatching. They are most marked, as shown by denser cross-hatching, in relation to the dorsal segment of the right atrioventricular orifice, but are also present in relation to the right-dorsal and right segments of the right atrioventricular orifice and the dorsal and left-dorsal segments of the left atrioventricular orifice. (*b*) The parts of the atrioventricular junctional region found by Lowman & Laurens (1924) to be important for coordinating atrioventricular rhythm are indicated by stippling. The most important, indicated by denser stippling, were the right-lateral segment of the right atrioventricular junction and the left-lateral and left ventro-lateral segments of the left atrioventricular junction. It will be seen that they do not correspond with the disposition of the muscular connexions shown in (*a*).

The most important differences between the findings of Swett and those here described are that: first, while Swett described muscular continuity of both atria with the dorsal part of the ventricular septum, the present work indicates that the dorsal wall of the left atrium is separated from the ventricular septum by dense fibrous tissue and that only the right atrium establishes direct muscular continuity with the ventricular septum; and secondly, that the direct muscular continuity between the atrial and ventricular septa described by Swett could not be substantiated.

Finally, there is the question whether the morphological features described above support the conclusions drawn by Lowman & Laurens (1924) from their experiments. Text-fig. 1 indicates the arrangement of the muscular connexions between the atria and ventricles in the heart of *Alligator mississippiensis* here described (*a*), compared with the results obtained by Lowman & Laurens (*b*) after cutting various segments of the atrioventricular junctional regions and noting their effect on atrioventricular co-ordination. The segments which, on cutting, they found to produce the most marked atrioventricular dissociation do not correspond with those in which muscular atrioventricular continuity was noted both by Swett and ourselves to be best developed. Indeed, they claimed that one of the least important segments for atrioventricular conduction was situated dorsally, where we found the muscular continuity to be most marked. It is concluded that the results of these experiments fail to substantiate the arrangements of the muscular connexions, and it is suggested that the severe injuries inflicted on the heart in these experiments may cause upset of the function of the heart that may not be a reliable guide to the normal paths of conduction of the impulse for cardiac contraction from atria to ventricles.

SUMMARY

1. In the hearts of *Alligator mississippiensis* and *Crocodilus niloticus* the musculature of the several chambers and that connecting one chamber to the next have a uniform histological structure, and no specialized nodal tissue or Purkinje fibres were found in any part of the hearts.

2. The sinus venosus is continuous with the right atrium all around the sinu-atrial orifice, the continuity involving the musculature of the sinu-atrial valves.

3. The right atrium is continuous with the right ventricle in the dorsal (principally), right-dorsal and right segments of the atrioventricular funnel, while continuity of the left atrium and left ventricle is limited to the dorsal and left-dorsal segments of the funnel.

4. The advent of a ventricular septum in the crocodilian heart has resulted in the establishment of a substantial muscular connexion between the right atrium and the dorsal part of the ventricular septum, but there is no direct muscular continuity between the left atrium and ventricular septum or between the atrial and ventricular septa.

5. It is believed that the muscular connexion between the right atrium and ventricular septum is not the homologue of the atrioventricular node and bundle of the mammalian or avian heart.

6. It is suggested that the observed pause between the contraction of the sinus and atrium and that between the systole of atria and ventricles are due to the circular arrangements of the muscle fibres proximal to each junctional site (sinu-atrial and atrioventricular 'rings').

We are indebted to our technical assistant, J. H. Kugler, who prepared the sections and photomicrographs. The expenses involved in the work were defrayed by a grant from the Medical Research Council to one of us (F. D.) and grants from the University of Sheffield Medical Research Fund, and for these we express our thanks.

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EXPLANATION OF PLATES

(All the illustrations are of the heart of *Alligator mississippiensis.*)

PLATE 1

Fig. 1. Photograph of ventral aspect of heart. × 3.

Fig. 2. Photograph of dorsal aspect of heart. × 3.

Fig. 3. Photograph of right side of heart. × 3.

Fig. 4. Transverse section of heart to show continuity between the right atrium and the ventricular septum, and the fibrous tissue separating the left atrial muscle from that of the ventricular septum. Mallory. × 6·2.

Fig. 5. Part of fig. 4 to show the same features more clearly. Mallory. × 14·5.

PLATE 2

Fig. 6. Transverse section through the sinu-atrial orifice and the bases of the aortae and pulmonary arch. Mallory. × 6·2.

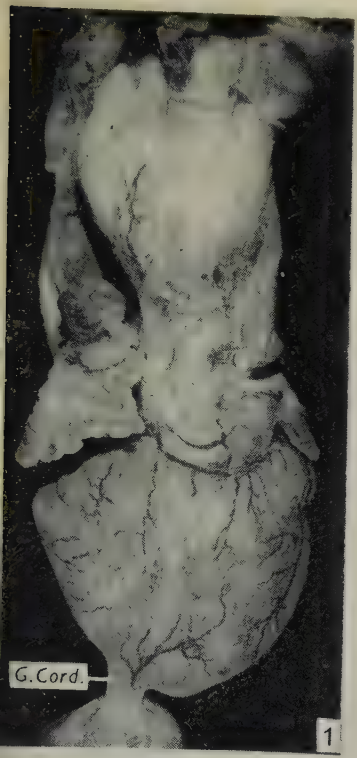
Fig. 7. Part of fig. 6 to show details of the sinu-atrial ring and sinu-atrial valves. Mallory. × 14.

Fig. 8. Transverse section through heart, just caudal to the sinu-atrial orifice, to show the foramen of Panizza between the left and right aortae and the bulbar muscle encircling the base of the pulmonary arch. Mallory. × 6·2.

Fig. 9. Frontal section through the atrioventricular junction to show the atrioventricular funnel and atrioventricular 'ring'. Silver-pyridine. × 22.

Fig. 10. Frontal section through the atrioventricular junction to show details of the left part of the atrioventricular funnel and of the atrioventricular 'ring'. Silver-pyridine. × 35.

Fig. 11. Frontal section through the atrioventricular junction to show the right septal cusp with its attached muscle, and the 'incipient' muscular cusp on the marginal aspect of the right atrioventricular orifice. Haematoxylin and eosin. × 16.



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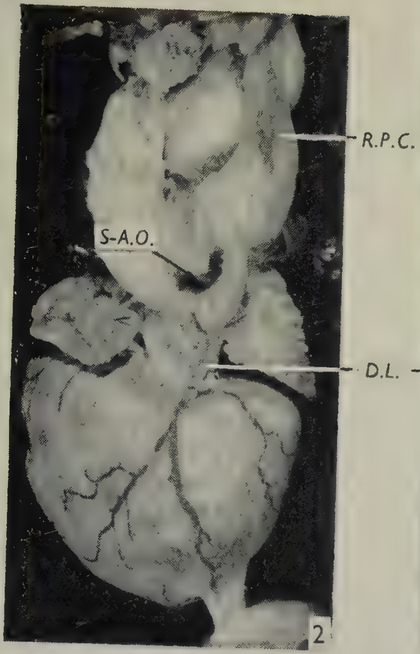

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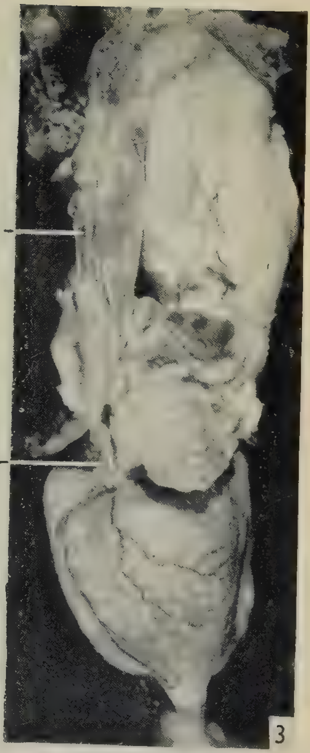

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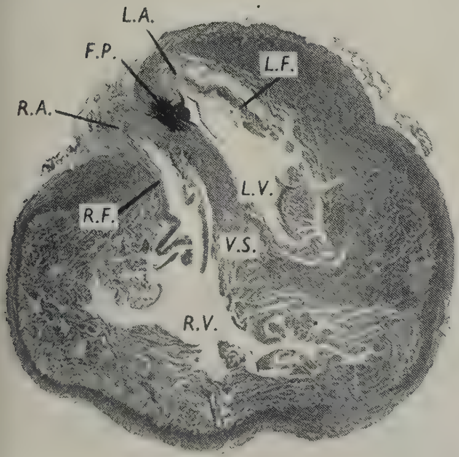

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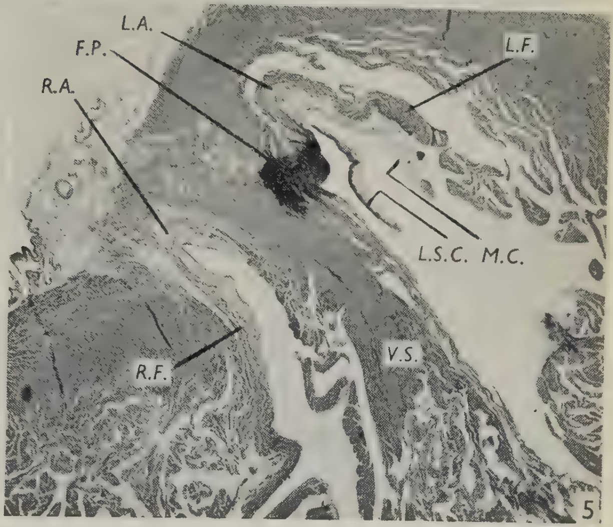

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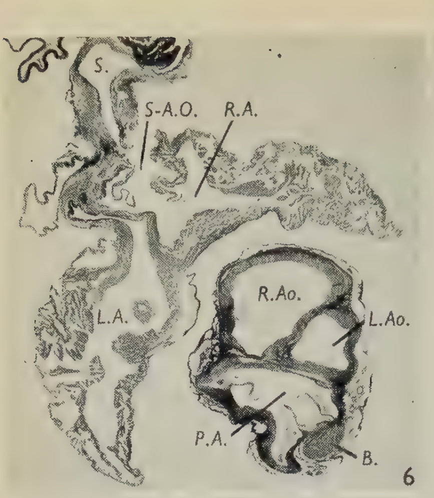
AVIES, FRANCIS AND KING—THE CONDUCTING (CONNECTING) SYSTEM OF THE CROCODILIAN HEART



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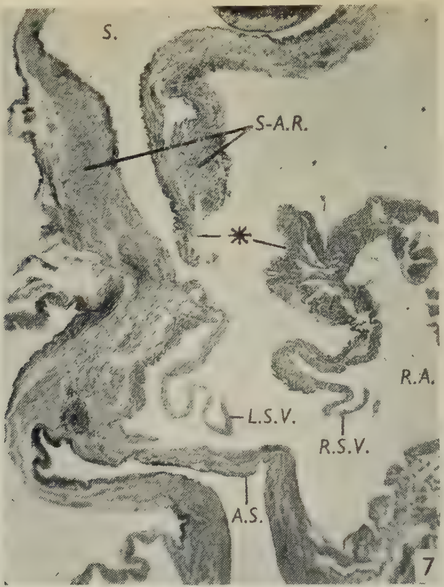

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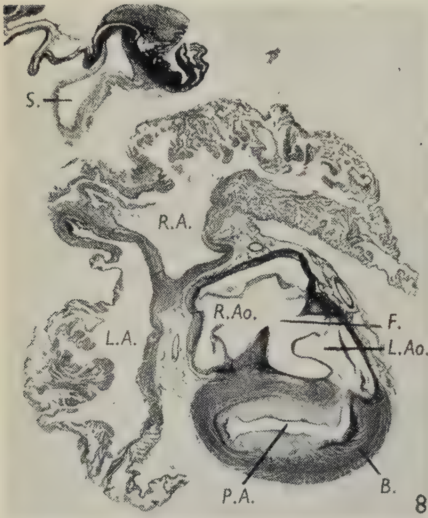

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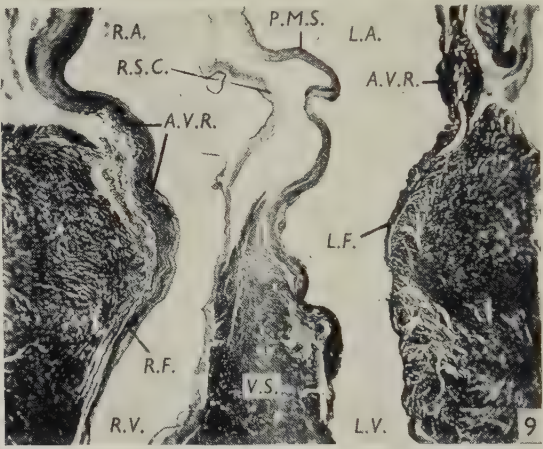

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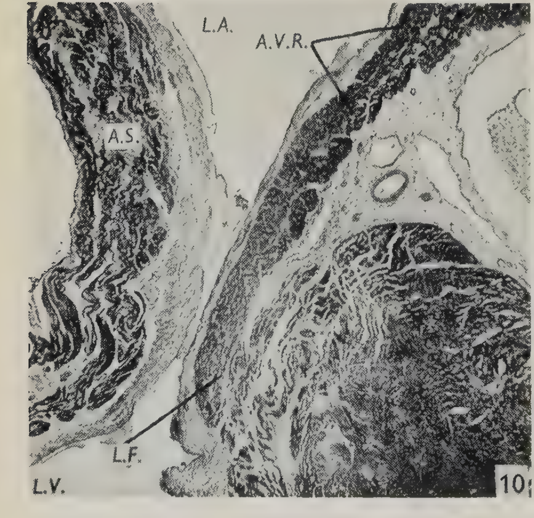

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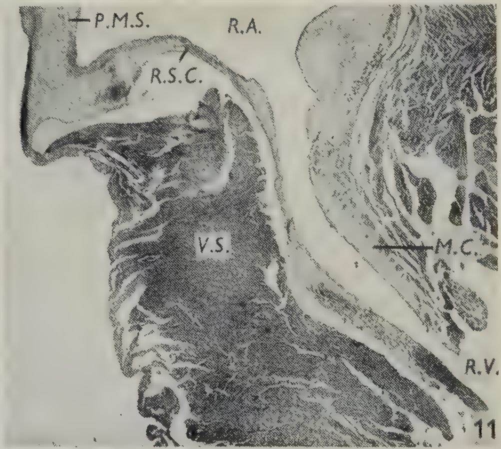

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DAVIES, FRANCIS AND KING—THE CONDUCTING (CONNECTING) SYSTEM OF THE CROCODILIAN HEART

LIST OF ABBREVIATIONS IN TEXT-FIGURE AND PLATES

A.S.	Atrial septum
A.V.R.	Atrioventricular ring
B.	Bulbar muscle
D.L.	Dorsal ligament
F.	Foramen of Panizza
F.P.	Fibrous tissue separating muscle of the left atrium from that of the ventricular septum.
G.Cord.	Gubernaculum cordis
L.A.	Left atrium
L.Ao.	Left aorta
L.AV.O.	Left atrioventricular orifice
L.F.	Left atrioventricular funnel
L.S.C.	Septal cusp of left atrioventricular valve
L.S.V.	Left sinus valve
L.V.	Left ventricle
M.C.	Marginal cusp of right atrioventricular orifice
P.A.	Pulmonary arch
P.M.S.	Pars membranacea septi
R.A.	Right atrium
R.Ao.	Right aorta
R.AV.O.	Right atrioventricular orifice
R.F.	Right atrioventricular funnel
R.P.C.	Right precaval vein
R.S.C.	Septal cusp of right atrioventricular valve
R.S.V.	Right sinus valve
R.V.	Right ventricle
S.	Sinus venosus
S-A.O.	Sinu-atrial orifice
S-A.R.	Sinu-atrial ring
V.S.	Ventricular septum
*	Artificial break at the base of the right sinus valve.

OBSERVATIONS ON THE POSTNATAL STRUCTURE OF THE INTERVERTEBRAL DISC IN MAN

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INTRODUCTION

The human intervertebral disc has long attracted the attention of anatomists. The earlier descriptions referred only to the macroscopic appearance. Later, with the development of improved technical methods, its microscopic structures received consideration. Vesalius (1543) referred to and figured the discs *in situ*, Bartholinus (1668) described these structures as 'gristles', and Winslow (1776) gave a more detailed account of the macroscopic appearance of the disc. Luschka (1856) described the anatomy of the discs at the extremes of life, and in his monograph on the hemiarthroses (1858) gave a detailed description of the macroscopic and microscopic structure and again referred to the changes seen at birth and in old age. Humphrey (1858) designated these structures 'intervertebral substances', described the arrangement of the fibres in the annulus fibrosus, noted the relative avascularity of the disc tissue and commented on the varying thickness of the discs in the different regions of the vertebral column. Henle (1872), Poirier & Charpy (1899), Fick (1904) and Petersen (1930) give good, well-illustrated descriptions of the disc with some reference to age changes. Schaffer (1910, 1930) refers to the disc in connexion with his studies of the notochord, Übermuth (1929, 1930) describes conditions present in certain decades, and Böhmig (1930) describes the structure in certain age groups in his studies of the vascularization of the disc. The monograph of Beadle (1931) is devoted to detailed descriptions of the macroscopic and microscopic anatomy of the disc, and to changes in the structure of the discs connected with certain pathological conditions of the spinal column. In the text-books of anatomy in general use the macroscopic appearance of the disc is described in very general terms.

A few only of the foregoing descriptions apply to discs of known ages. In most of the accounts given, no reference is made to the age of the specimen described. To obtain a comprehensive idea of the changes occurring in the tissue components of the disc, a wide range of specimens of known ages is required for examination.

The structure of the disc at a few known ages is given by Smith (1931), Keyes & Compère (1932), and more detailed descriptions of the structure of the lumbosacral disc in the various decades are given by Coventry, Ghormley & Kernohan (1945). Amprino & Bairati (1934), in their studies of age changes in fibrocartilage, employed the intervertebral discs as one of the subjects for study, and Franceschini (1947) has investigated the changes in the arrangement of the fibres of the annulus fibrosus at different ages, by examination with polarized light.

The present investigation was undertaken, following a previous study of the prenatal changes in structure of the developing intervertebral disc, to provide data for a survey of the life history of the disc. Attention was particularly directed to the changes occurring in the nucleus pulposus.

MATERIALS

Thirty fresh post-mortem specimens of lumbar discs were obtained from subjects of known ages, ranging from full term to 80 years of age, and, as far as known, free from spinal disease or deformity. The specimens were examined macroscopically and microscopically. For microscopic examination serial sections of some complete discs were made; in other discs, a strip was taken from the middle of the disc in the sagittal plane, thus including the nucleus pulposus, and then serially sectioned. Some specimens obtained by operation from patients with protrusion of the lumbar discs were also examined for comparison.

DESCRIPTION OF SPECIMENS

The specimens described are selected as showing well-marked stages of the structural changes in the tissue components of the disc. The marked pathological changes (haemorrhage, pigmentation) occurring with increasing frequency, in ageing discs, are not considered.

At full term

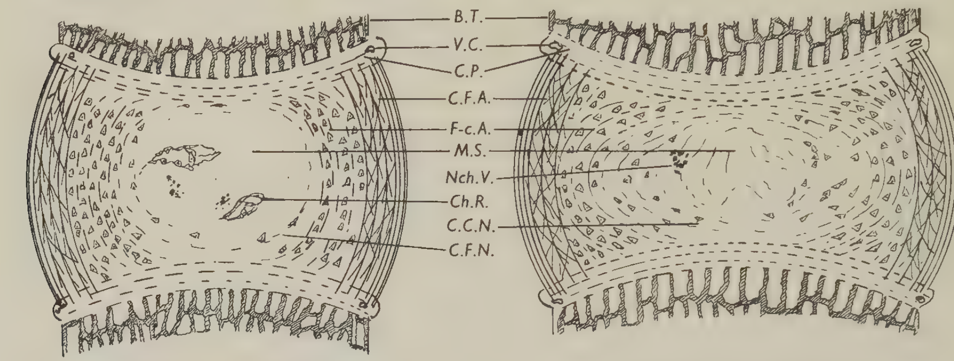
In the photograph, two vertebrae with the intervening disc are seen (Pl. 1, fig. 1; Text-fig. 1). The cartilage plates capping the end-surfaces of the vertebral bodies are clearly defined, and in the disc, the inner nucleus pulposus and the outer annulus fibrosus are seen. The disc, as a whole, is biconvex and of considerable thickness, the vertical height being almost half that of the adjacent vertebral bodies. A noteworthy feature is the size of the nucleus pulposus, which occupies in this plane half the antero-posterior diameter of the whole disc. The shape of the nucleus pulposus is quadrilateral, and it appears to be composed of an apparently homogeneous substance containing scattered clumps of cells. The annulus fibrosus exhibits an outer area of densely aggregated fibres and a less dense area, between the former area and the nucleus pulposus. In both these areas, arching fibres may be seen, which, at their terminations, enter the cartilage plates.

On microscopic examination the periphery of the annulus shows a dense aggregation of collagenous fibres, which run across the intervertebral space, following an outwardly convex course. The terminations of the fibres are lost in the matrix of the cartilage plates, to which they are firmly anchored. Apart from the most peripherally situated lamellae, these fibres do not follow a simple course, but splitting of some fibre-bundles occurs with subsequent joining with those of other bundles; some fibres pursue a spiral course across the intervertebral space, and the interlocking and crossing of the fibres forms an intricate system (Pl. 1, fig. 2).

Within this area of the annulus is the less dense area previously referred to. This is a zone of fibrocartilage, the fibres of which follow an arching, outwardly convex course. Within this zone again is the nucleus pulposus, the distinction between the two areas being clearly demarcated. There is, however, a narrow transitional zone.

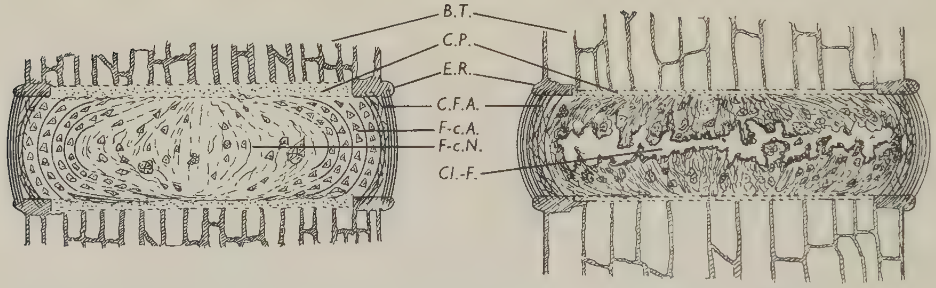
The nucleus pulposus is formed by a mass of homogeneous, mucoid substance, in which are seen at intervals the remains of a chorda-reticulum (Pl. 1, fig. 3). The chorda-reticulum is formed by a meshwork of notochordal cells, separated by intercellular spaces containing mucoid substance, some cells being compressed into long tracts, others being isolated as small clumps. Typical notochordal cells are recognizable, but many cells show nuclear pyknosis and fragmentation. This area may be termed the 'notochordal' area.

The narrow transitional area referred to above exhibits the following features. The innermost area of the fibrocartilaginous component of the disc appears to be undergoing liquefactive changes, resulting in the separation of cartilage cells and fibres from this area and their mingling with the mucoid substance at the periphery of the 'notochordal' area. Similar changes, though much less obvious, appear to be occurring at the faces of the cartilage plates bounding the nucleus pulposus above and below. Centrally the intervertebral surfaces of these plates seem to be well defined, but, more peripherally, fibres running parallel to the surface of the plate arch to mingle with those of the fibrocartilaginous component.



Text-fig. 1. At full-term.

Text-fig. 2. At 10 years.



Text-fig. 3. At 33 years.

Text-fig. 4. At 80 years.

Text-figs. 1–4. Diagrammatic representation of age changes in the tissues of the disc.

List of Abbreviations

B.T.	Bone trabeculae	*E.R.*	Epiphyseal ring
C.C.N.	Cartilage cells in the nucleus pulposus	*F-c.A.*	Fibrocartilage of annulus fibrosus
C.F.A.	Collagenous fibres of annulus fibrosus	*F-c.N.*	Fibrocartilage of nucleus pulposus
C.F.N.	Collagenous fibres in the nucleus pulposus	*M.S.*	Mucoid substance
Ch.R.	Chorda-reticulum	*Nch.V.*	Notochordal vestige
Cl.F.	Cleft-formation	*V.C.*	Vascular canal

Small blood vessels are present between the lamellae of the annulus fibrosus in the postero-lateral regions of the disc, but the nucleus pulposus is avascular.

In specimens of the ages of 3, 17 and 21 days the structural features are as described at full term.

At 5 weeks

The general features are much the same as at birth (Pl. 1, fig. 4), the components of the disc from without inwards being, the zone of collagenous fibres, the fibrocartilaginous area, the transitional area and the 'notochordal' area.

The nucleus pulposus occupies a large area relative to the total area of the whole disc, but the thickness of the disc compared to the height of a vertebral body is less than in the previous stages. There is an alteration in the shape of the nucleus pulposus, which has now assumed a more oval form as seen in the median sagittal plane.

A fairly sharp demarcation exists between the nucleus pulposus and the surrounding fibrocartilage, the transitional area being very narrow. This latter area again shows merging of the tissue components of the inner aspect of the fibrocartilaginous area with the 'notochordal' element. In the more central parts of the 'notochordal' area, mucoid substance containing small clumps of notochordal cells is present, many of these cells showing signs of degeneration. The nucleus pulposus is avascular.

Similar conditions are present at 5 months of age.

At 4 years

On macroscopic examination, the annulus fibrosus and nucleus pulposus are clearly defined, and the latter occupies a considerable part of the total area of the whole disc.

On microscopic examination the outer collagenous fibres, the fibrocartilaginous zone, the transitional area and the 'notochordal' area are seen, but this latter area shows further changes. There is abundant mucoid substance in this area which now contains a loose irregular network of fine collagen fibres. At intervals in the notochordal area, remains of a chorda-reticulum are present and isolated clumps of notochordal cells, many of which are degenerated (Pl. 1, fig. 5). More cartilage cells are seen in the peripheral area of the nucleus pulposus. The nucleus pulposus remains avascular.

At 10 *years*

Microscopically the same tissue components as previously described are present. The nucleus pulposus is still relatively very large and occupies about half the area of the disc. In this area abundant mucoid substance is present, more collagenous fibres are present and small groups of cartilage cells are seen scattered in the mucoid substance (Pl. 1, fig. 6; Text-fig. 2). Remains of a chorda-reticulum are still present, but most of the cells are degenerated and very few typical notochordal cells are recognizable.

No blood vessels enter the nucleus pulposus.

At 21 *years*

Macroscopically, the annulus and the nucleus pulposus are clearly demarcated, but the distinction is not so pronounced as in the previous stages. The tissue of the nucleus appears less translucent, and though it is of markedly gelatinous character, it is of firmer consistency.

Microscopically, the tissue zones previously described can be recognized, but the innermost zone can no longer be described as 'notochordal'. This area is now occupied by a delicate, irregularly arranged fibrocartilaginous network containing much mucoid substance. The cartilage cells are scattered through this tissue sometimes singly or in small groups, but occasional nests of cells are present (Pl. 2, fig. 7). The nucleus pulposus is avascular.

At 33 *years*

With the naked eye annulus and nucleus pulposus can be defined, but the distinction is less well-defined than in earlier stages. The nucleus has lost its translucent appearance, and appears whitish, is of firmer consistency and may be described as fibrogelatinous.

On microscopic examination the fibres of the annulus have become coarser and somewhat hyalinized. In the nucleus the fibrocartilage network shows coarser fibres and cartilage cells are more numerous. The cells are scattered or present in small groups and more nests of cells appear (Pl. 2, fig. 8; Text-fig. 3). Mucoid substance is still present in the matrix, but the whole tissue appears to have lost fluid and to have become denser. The nucleus pulposus remains avascular.

At 40 *years*

The annulus fibrosus and the nucleus pulposus can still be distinguished, though the demarcation is becoming progressively less obvious. The nucleus pulposus appears whitish and is of firm consistence.

Microscopically, there is little change in the annulus fibrosus, but the nucleus pulposus again shows irregularly arranged fibre bundles and numerous cartilage cells (Pl. 2, fig. 9), nests of cartilage cells being more numerous. The whole fibre meshwork appears denser and shows evidence of loss of fluid. No vessels enter the nucleus.

At 50 *years*

There is little change in the general appearance of the disc. Annulus fibrosus and nucleus pulposus can still be distinguished. Microscopically, the fibres of the annulus show hyalinization and coarsening. The tissue of the nucleus pulposus is now a fibrocartilage and very like the surrounding tissue, but the fibre bundles are less closely aggregated and there is no comparable fibre architecture, the bundles of fibres coursing indiscriminately. Numerous cartilage cells are present and nests of cartilage cells are frequently seen (Pl. 2, fig. 10). No vascularization of the nucleus pulposus is seen.

At 64 *years*

The distinction between the nucleus pulposus and the annulus fibrosus is not very clear macroscopically. Microscopically, hyalinization of the fibres of the annulus is present, but there is still evidence of a definite fibre architecture. The nucleus pulposus is composed of a dense fibrocartilage which shows the characteristics described for the previous stage (Pl. 2, fig. 11). Masses of amorphous substance are found at places between the fibre bundles. The nucleus pulposus is avascular.

At 75 *years*

Macroscopically, distinction between the nucleus pulposus and the surrounding tissues is difficult, but the nucleus still has a softer consistency than the perinuclear components. In the low-power photograph, cleft formation is seen in the disc, horizontal fissuring extending almost completely through the disc (Pl. 2, fig. 12).

Microscopically, there is little change in the tissues surrounding the nucleus pulposus. The nucleus itself is a coarse fibrocartilage (Pl. 3, fig. 13) and is avascular.

At 80 *years*

On macroscopic examination, conditions are much as those described in the 75-year-old stage. The whole intervertebral mass appears as a uniform plate of tissue, and distinction between the nucleus pulposus and the annulus fibrosus is lost. Cleft formation is again seen in the disc (Pl. 3, fig. 14; Text-fig. 4).

Microscopically, the peripheral layers of collagen fibres are still seen arching, with outward convexity, between the adjacent vertebrae. These fibres are hyalinized, the sharp definition of their structure being lost. Within these, the fibrocartilaginous component of the disc is seen, the component fibres are hyalinized, and the cartilage cells appear indistinct, but fibres can be traced into the thin cartilage plate covering the upper and lower surfaces of the vertebrae. Where the terminations of these fibres merge with the matrix of this cartilage a very narrow transitional zone is occasionally recognizable. Towards the periphery, at the outer borders of the vertebrae, the cartilage plates appear to be absent and the fibre terminations enter the bone directly.

More centrally in the disc, the area of the nucleus pulposus can still be recognized by the indiscriminate course of the fibre bundles and by their less close aggregation. Numerous cartilage cells are present between the fibre bundles and nests of these cells are of frequent occurrence. The fibre bundles show lack of definition and appear hyalinized like those of the perinuclear components (Pl. 3, fig. 15). The nucleus is avascular.

Cleft formation in the disc is a marked feature, both horizontal and vertical clefts being present. The horizontal cleft formation passes almost completely through the disc from the anterior to the posterior surface, but some fibres are present in this outer region, and beyond these are the fibres of the longitudinal ligaments. The vertical clefts in some instances result in the appearance of processes resembling villi.

Specimens obtained by operation

Specimens obtained from patients with disc lesions between 20 and 40 years of age, examined microscopically, show structural features similar to those described above. They contain tissue from the nucleus pulposus showing delicate fibrocartilage containing mucoid substance and exhibiting nesting of cartilage cells, and fibre aggregations from the outer annulus components. Occasionally parts of the cartilage plates and fragments of the bone of the vertebra are seen.

It may be remarked that considerable individual variation occurs in the structure of the intervertebral disc, in the sense that some discs appear to have aged more quickly than others. This is in accordance with the known individual variability of age changes in the body generally.

Review of observations and discussion

(1) *The tissue-components of the intervertebral disc*

The descriptions of the constitution of the intervertebral disc, as usually given, require consideration. The disc is commonly described as consisting of two components, an outer firm annulus fibrosus, and an inner soft nucleus pulposus. To these components, some authors add the cartilage plates, which, capping the end-surfaces of the vertebral bodies, bound the intervertebral area cranially and caudally. In this connexion, it may be noted that in early stages of development, the tissue intervening between the developing cartilaginous vertebral bodies is frequently referred to as the 'perichordal disc' (Dawes, 1930; Wyburn, 1944). This perichordal disc presents three areas, a cranial, a middle, and a caudal area. The middle component of dense mesenchyme is the *anlage* of the intervertebral disc, while extension of chondrification of the vertebral bodies invades the less dense cranial and caudal mesenchymal areas, which are the primordia of the cartilage plates. Thus, some authors (Prader, 1947) consider the cartilage plates as parts of the vertebral bodies, while others regard them as parts of the disc. In the present description they are considered as 'vertebral' and referred to only as far as they are concerned in the anchoring of the disc fibres, attention being primarily directed to the soft tissues.

The description of the disc as comprising an annulus fibrosus and a nucleus pulposus is somewhat simplified. From the observations in the present investigation, it is seen that, traced horizontally from the periphery of the disc towards the centre, the tissue-components are (1) the narrow outermost zone of collagenous fibres, within this (2) the wider zone of fibrocartilage, followed by (3) a transitional area between the latter, and (4) the nucleus pulposus. The transitional area varies with age. In the earlier periods it is narrow and separates tissues with very different structural features, so that the distinction between annulus and nucleus is very obvious. Later, with the subsequent transformation of the structure of the nucleus pulposus and the merging of this tissue with that of the perinuclear tissue, the transitional area is difficult to define.

If the terms in common use are to be retained, the disc may be described as consisting of an outer annulus fibrosus, and an inner nucleus pulposus. The annulus fibrosus exhibits a narrow peripheral zone of collagenous fibres and an inner zone of fibrocartilage. The structure of the nucleus pulposus varies markedly with the advance of age. The various components of the disc may be considered separately.

(2) *The annulus fibrosus*

(*a*) *The outer collagenous component.* The course of these fibres at the periphery of the disc may be traced by simple dissection, and the obliquity of the course of many superficial fibres and the crossing with fibres running in the opposite direction, clearly demonstrated. In the lumbar region, fibres arising from the postero-lateral region of the discs may be shown to pass caudally and laterally, where they fan out to reach and become attached to the pedicles of the next subjacent vertebra. These fibres thus form the anterior, and, in part, the inferior boundary of the outer opening of the intervertebral canal. Other fibres may be traced from this area of the disc, passing caudally and anteriorly to join the periosteum of the subjacent vertebra near its lower border, or to blend with those of the subjacent disc, crossing in this course the lumbar vessels. Fasciculi are frequently seen passing between the lumbar arteries and veins.

In a sagittal section of the disc, examined microscopically, these oblique fibres are sectioned transversely at various angles, while a series of vertically running fibres is sectioned longitudinally. Deep and superficial, obliquely running fibres, intersecting the vertical fibres, may be observed. The vertical fibres show an arched course with an outward convexity, are closely aggregated and mingle with the fibres of the longitudinal ligaments, the connexion being more intimate anteriorly. With the advance of age, all the collagenous fibres undergo hyalinization but show no further changes.

(*b*) *The fibrocartilaginous component.* This is the principal component of the annulus fibrosus, and in it the fibre architecture is of an intricate character. Concentric and vertical systems of fibres are present, and the splitting and joining of adjacent fibre bundles result in a complicated meshwork. On macroscopic examination the upper or lower surface of a disc presents somewhat the appearance of damask, the patterns running concentrically. Fick (1904) refers to this and states that the earlier authors thought that the pattern and slight differences in colour observed represented different component tissues. Henle (1872) was apparently the first investigator to point out that only one tissue was concerned and that these phenomena were due to the differing courses of the fibres, causing an unequal reflexion of incident light.

Übermuth (1929, 1930) considers that the formation of the fibrocartilage component occurs only with the advent of mechanical stresses associated with the sitting up of the infant at 6 months after birth and with walking, and regards it as an age change. Böhmig (1930) associates the formation of this component with the presence of vascularization. In referring to vessels which he states are present in the disc, he states: 'Die sind es ferner, die die Fasern des Annulus lamellosus zu bilden und liefern scheinen und dessen verflochtene Fasersysteme entstehen lassen', and refers to a development of the fibres from the vessels of the disc thus: 'Diese Fasern scheinen von allen Baudscheibe-Gefässen, besonders aber den axialen, auszugehen, wodurch die eigentümliche Verlaufsrichtung der Fasersysteme des späteren Annulus lamellosus ihre Erklärung findet.' It has, however, been shown (Peacock, 1951) that very early in development (at 10 mm. c.r.) orientation of the mesenchymal cells occurs, that peripheral fibres develop by the 29 mm. stage, and that the fibrocartilaginous component is derived from a specialized embryonic cartilage directly surrounding the notochord. Further, these processes occur before vessels are present in the disc and the complex fibre architecture develops before any mechanical stresses are encountered.

The principal age changes occurring in this component are closer aggregation of the fibres during the second decade, and with the third decade the onset of coarsening and hyalinization of the fibres, nesting of the cartilage cells, some of which begin to show signs of degeneration, desiccation and cleft formation. From this period on, such changes are slowly progressive.

The disc participates in the general growth processes occurring in the body and in this connexion attention may be directed to the mode of termination of the fibres in the cartilage plates. The ends of the fibres are lost in the matrix of the cartilage of the plates (Pl. 3, fig. 16). This area is especially referred to by Kaufmann (1854) and Amprino & Bairati (1934), the latter authors designating it as a transitional zone and pointing out that during the active growth of the disc this area is a prominent structural feature. The fibrocartilaginous area increases at the expense of the cartilage of the plates. Coventry *et al.* (1945) note that after the age of 14 to 16 years proliferative processes in the plate are much less active, and that by the age of 20 years there is little evidence of endochondral growth. With the extension of ossification of the vertebral body, the cartilage plates become very thin, and coincidently with this, growth in thickness ceases in the intervertebral disc.

In the adult, the outermost circumferential fibres of the annulus are embedded in the so-called 'epiphyseal ring', which is developed from the cartilage plate. The method of its development may be briefly described. Frazer (1940) refers to ossification taking place in the periphery of the cartilage plate. Schmorl (1928*b*, *c*), in describing the special characteristics of juvenile vertebrae, gives a much more detailed account. Macerated juvenile vertebral bodies exhibit radially arranged furrows on the cranial and caudal vertebral end-surfaces, which, deepening as they approach the periphery, notch the borders of the vertebral bodies. Prior to maceration, these furrows are occupied by corresponding processes arising from the apposed surfaces of the cartilage plates. In the peripheral areas of these cartilaginous processes, small centres of ossification appear from as early as 8 years of age, and thus a peripheral ring of small centres of ossification develops. Later, these centres coalesce to form the 'epiphyseal ring', which finally fuses with the vertebral end-surface, with the cessation of growth. Even after complete fusion, persistent juvenile furrowing may occasionally be seen on the end-surfaces of adult vertebrae, within the epiphyseal rings. Schmorl considers that these epiphyseal rings serve a special function, in acting as an anchoring mechanism for the outer fibres of the annulus. These rings are indicated in Text-figs. 3 and 4.

As regards the growth of the disc, in girth, it may be noted that in the first decade, the nucleus pulposus is almost centrally placed. Later, the nucleus appears to approach the posterior aspect of the disc. This is due to the fact that the disc and vertebral body do not increase in girth concentrically, the increase taking place by apposition, on the anterior aspect of these structures. The growth of the disc in the thoracic and lumbar regions follows that of the vertebral bodies, the mode of growth of which has been shown radiologically by Knutsson (1948).

In connexion with the growth processes occurring in the disc, it may be noted that the discs of the cervical region show certain special features. Whereas the thoracic and the lumbar discs throughout prenatal and postnatal life occupy the whole area of the vertebral end-surface, the cervical discs in prenatal and early postnatal life are confined to the area of the true centrum of the vertebral body. This centrum is clearly demarcated from the vertebral-arch component of the vertebral body by the cartilage of the neurocentral synchondrosis. Bony fusion begins 3 or 4 years after birth, though evidence of the line of union remains for some years after this. Information regarding the peculiarities of the lateral areas of the cervical discs has accumulated as a result of studies directed to the so-called Luschka's, neurocentral or unco-vertebral joints present in the cervical vertebral column. Luschka (1858), in describing the connexions of the cervical vertebrae, states that (excluding the atlas and axis) three joints (a middle and two lateral) are present, between the end-surfaces of the cervical vertebrae. The lateral joints possess a clearly defined cavity, well demarcated from the intervertebral disc. He states that these cavities may be occupied by connective tissue, a condition regularly found in the newborn, but that in later years, as a result of fissuring in the nucleus pulposus and annulus fibrosus, connexion may occur between the lateral joints and such fissures. He refers to the fact that the annulus does not encircle the whole vertebral connexion, but extends laterally on the vertebral body to be limited by the medial limit of the lateral joint. He refers to the presence of synovial fluid and villiform processes of connective tissue, in the cavities of the lateral joints. Many authorities have since assumed that these joints are synovial and they are often described as such. Rathcke (1934) reports that he does not find true joint cavities present, but interprets these formations as tears in the disc. His Fig. 1, of a disc of a newborn child illustrated by Rathcke, clearly shows the extent of the disc in the horizontal plane; in Fig. 2 the lateral extension, together with cleft formation, is seen in a specimen from a 20-year-old man. Krogdahl & Torgersen (1940) find crevices laterally placed in the cervical region, in the sites described by Luschka. They consider the medial boundary to be formed by the disc, but question the propriety of designating these formations as true joints. Töndury (1943) has made investigations employing a much wider range of material, both prenatal and postnatal, and finds that at birth and up till 7 years of age, the disc is confined to the centrum of the vertebra. At 9 years he finds a further lateral extension of the disc, beyond the centrum into the region of the uncinate processes or neurocentral lips. At the same time a cleft appears, present on both sides, which contains connective tissue including blood vessels and fat. The process of cleft formation is brought about by loosening, degeneration and rupture of the fibres in the lateral part of the annulus. This process is a regular feature in the cervical region, and the clefts present are the so-called Luschka's joints, though no joint space, in the sense of a synovial cavity, is present. Töndury considers this phenomenon of cleft formation to be the result of functional demands associated with the mobility of the cervical column. He has shown, in the case of a 24-year-old man, that the lateral clefts may extend to the nucleus pulposus, so that the disc is subdivided horizontally, very much as is seen in the 80-year-old specimen of a lumbar disc in this investigation.

The direction of certain of the fibre components of this fibrocartilaginous area are worthy of comment (Pl. 3, fig. 17). At the superior and inferior boundaries of the nucleus pulposus, at the opposing vertebral end-surfaces, as seen in the median sagittal plane, there are to be observed fibre bundles which run almost parallel to the surface of the vertebra, but which, as they reach the anterior of the posterior aspect of the nucleus pulposus, turn towards the opposite vertebra and mingle with the outwardly convex and vertically running fibres in the perinuclear area of the fibrocartilaginous component. These fibres are at first closely aggregated near the vertebrae. As they pursue their outward course, however, they become separated by mucoid substance continuous with that of the nucleus, and they and their accompanying cartilage cells merge with the tissue of the nucleus. This is a transitional zone between the cartilage plate of the vertebral body and the nucleus pulposus, but is less pronounced than the transitional area described below. With the advance of age and the transformation of the nucleus, these originally horizontally running fibres change their course and come to run almost vertically into the nucleus.

Other age changes, reflected in the course of the fibres, are referred to by Franceschini (1947, 1949). He examined specimens of discs from the cervical, thoracic and lumbar regions, at various ages, by polarized light. He states that in horizontal sections, all regions show the characteristic concentric arrangement of fibres, which remains fundamentally unchanged with the advance of age, but that birefringence, slight at 3–5 years, is accentuated at 13–15 years. As seen in frontal and sagittal sections the fibre orientation at 7–10 years in the cervical region is that of a series of superimposed parallelepipeds enclosing the nucleus pulposus, while in the dorsal and lumbar regions the orientation may be compared to that of an ovoid with a transversely placed major axis. This architecture changes with age, and frontal and sagittal sections in specimens of 18–20 years show that the major lamellae near the nucleus pulposus assume a complex configuration which he designates as 'a cuscinetto'. The vertical peripheral lamellae are succeeded by more internally situated lamellae arching with an inwardly directed concavity. These are succeeded by central lamellae, some vertical, some undulating, and finally next to the nucleus pulposus, there are other lamellae, arched but with the concavity facing outwards. This architecture appears early at 18–20 years in the lumbosacral region and later in the dorsal and cervical regions. With further advance of age the lamellar complex undergoes many variations, in response to mechanical demands. At 40–45 years he reports marked lateral projection of the outwardly arching lamellae, which he considers an anatomical basis for discal herniation. In the series investigated no regular pattern could be ascribed to the fibres of the transitional area between nucleus and annulus.

(3) *The transitional areas*

Two transitional areas have been previously referred to, one associated with the terminations of the fibres of the fibrocartilaginous component in the cartilage plates (Pl. 3, fig. 16), the other situated at the junctional area of the nucleus pulposus and the cartilage plates (Pl. 3, fig. 17). This latter area is similar in structural principle to that to be described, but is less evident.

The area now referred to is that seen at the periphery of the nucleus pulposus, between this and the fibrocartilaginous component of the annulus, and the structural features are shown in Pl. 3, fig. 18.

It may be defined as the area of the merging of the tissue of the 'notochordal' nucleus pulposus and the inner part of the fibrocartilaginous component. In this area of the fibrocartilaginous zone, a process of liquefaction of the matrix appears to be taking place, resulting in the separation of the fibres and cartilage cells which are seen passing into the periphery of the nucleus pulposus. Luschka reports strands of tissue derived from the perinuclear area projecting into the notochordal area, and compares them with 'synovial villi' as seen in the cavity of a synovial joint. The comparison is rather misleading, as the structure and formation of the structures concerned are different.

The tissue changes occurring in this region appear to be the first stages in the transformation of the nucleus pulposus, and have begun at birth. Thereafter they are progressive and, as the transformation of the nucleus is accomplished, the transitional zone becomes more difficult of definition.

(4) *The nucleus pulposus*

The structure of the nucleus pulposus has been a subject of controversy, the principal argument concerning the fate of the notochord and the part this structure plays in the formation of the nucleus pulposus.

There is an extensive earlier literature on this subject, which was summarized by Williams (1908). It is sufficient for the present purpose to state that two theories were held, one maintaining that the notochord degenerated and disappeared without trace, the other that it played a part of more or less significance in the formation of the nucleus pulposus.

From observations made in the present investigation, it may be concluded that a notochordal element is definitely present in the early postnatal stages, that this progressively diminishes in extent, and is progressively replaced by fibrocartilage with the advance of age.

The difficulty of definition of the structure of the nucleus pulposus arose from the fact that the earlier observers did not examine a sufficiently wide range of specimens of known ages, to show that transformation of the nucleus was taking place. Hence, their descriptions, in the main correct for the specimens examined, could not give an adequate picture of the changing structural features.

The latest age periods at which notochordal elements are present in the nucleus have been variously estimated by different authors. In fact, there are very few references to definite age periods recorded, the age period being referred to in general terms such as 'adult' or by decades. Schaffer (1910) stated that in a 30-year-old man, he could recognize no characteristic notochordal tissue, other than detritus, which he considered to represent the final vestiges of the epithelial chorda-cells of the newborn. In a later contribution (1930) this investigator figures and describes the nucleus pulposus in the newborn and from subjects of 19 and 28 years of age. In the 28-year-old subject, he states that 'of chorda-tissue, only granular detritus and large, homogeneous oxyphil scales are present, often joined into rounded masses by mucoid, while the surrounding cartilage of the disc shows softening, whereby whole territories of cartilage cells are isolated and may enter the chorda-cavity'. He concludes that, ultimately, in the adult nucleus pulposus the intimate mingling of products of degeneration of notochordal tissue, and of the surrounding cartilage, renders impossible the determination of the origin of individual cells and cell remains. Yet he states that in the nucleus of a subject of 67 years, he can recognize remains of chorda-cells, but considers that 'isogeneous encapsulated cell-groups are derived from cartilage', as Dursy (1869) had previously maintained. Keyes & Compère (1932) state that in a 4-year-old child, 'notochordal cells are difficult to find', while Amprino & Bairati (1934) suggest that after 15 years of age conspicuous masses of notochordal cells are no longer present, but that small groups may be found on careful examination. Coventry *et al.* (1945) report that they did not find typical notochordal cells in the specimen of 10 months of age. In the present series, remains of a chorda-reticulum and but few recognizable isolated chorda-cells are to be seen in the specimen of the 10-year-old subject, and after this age no chorda-vestiges are present.

Some difficulty has arisen over the identification of grouped cells. The older authors (Kölliker, 1879; Fick, 1904) refer to the presence of notochordal cells in 'adults', no specific age being given, and refer also to 'mother cells' which they assume to be of notochordal origin. Schaffer (1930) suggests an origin for these cells, as noted above, and Petersen (1930) has never found notochordal cells present in the adult and considers the presence of such cells to be 'very doubtful'. He considers that the vesicular cells described and illustrated by Fick (1904), and the older authors, are nests of cartilage cells with capsules. It has been pointed out by Amprino & Bairati (1934) that these structures must have originated from cartilaginous elements, for they are evidently late in making an appearance, and further these authors have observed similar cell groups in the symphysis pubis, in which, of course, no notochordal elements are ever present. From observations in the present investigation, it appears that the nested cells are cartilage cells.

Some further aspects of the question of persistence of notochordal tissue require consideration. Musgrove (1891) refers to the presence of notochordal tissue in an adult subject, connecting the nuclei pulposi above and below the 4th lumbar vertebra, by passing through the body of this vertebra. The condition was discovered fortuitously. The microscopic findings reported are not very convincing, and there is some doubt as to whether true notochordal tissue was present. Schmorl (1928*a*), from an investigation of 3000 vertebral columns, reports the presence of 'Chordareste' as frequent, and that these may be present in the bodies of the vertebrae. He states that true notochordal cells were observed microscopically.

In examining a range of serially sectioned human embryos, abnormalities of the notochord are not uncommonly observed. Branching of notochordal tissue from the main notochordal column with protrusions extending in variable directions, both into the intervertebral discs and into the vertebral bodies, may be observed. Fragmentation of the notochord may be present, with the detachment of small islands of notochordal tissue, and their location in the discs or vertebral bodies. Partial duplication of the notochord has been described by Johnston (1931).

Such embryonic 'rests' may provide the basis for the development of chordomata. Spheno-occipital and sacro-coccygeal chordomata are well-known clinical and pathological entities. Chordomata affecting the cervical, thoracic and lumbar vertebrae are less common, but Cappell (1928), Davis & Weil (1928), Machulko-Horbatzewitsch & Rochlin (1930), Mabrey (1935), Faust, Gilmore & Mudgett (1944), and Morris & Rabinovitch (1947) all record such cases. Excluding sacro-coccygeal chordomata, vertebral chordomata are mainly found in the cervical region (Adson, Kernohan & Woltman, 1935), while lumbar chordomata are next in frequency.

Hence, embryonic notochordal tissue may persist in certain instances, and may either remain quiescent or develop neoplastic characteristics. In the normal course of events, the fate of the notochordal cells appears to be one of progressive diminution in number associated with retrograde changes and final disappearance.

The nucleus pulposus has considerable power of imbibition of fluids, far in excess of that of the annulus fibrosus. With advancing age, desiccation of the disc is progressive. Investigations of the water content of the disc at various ages, by Püschel (1930), Keyes & Compère (1932), and Schümmelfeder & Schümmelfeder (1949) show very similar results. Püschel finds the highest values for the newborn, both for the annulus (77 %) and the nucleus (88 %). At 18 years, the values are 72 and 80 % respectively. In the third decade, the annulus reaches a value of 70 % which it retains till old age, when it decreases slightly, the nucleus, however, undergoing a steady decrease. Thus, the difference between annulus and nucleus progressively decreases. At 77 years, Püschel finds the value for the annulus to be 67 % and for the nucleus 69 %. The changes are closely paralleled by the structural changes occurring at the corresponding age periods, as observed both macro- and microscopically. The decrease in height of the discs resulting from the loss of volume is shown by the measurements of Jacobi (1927), and from comparisons of such measurements in the newborn and adult given by Hasselwander (1938). The desiccation occurring in the disc probably plays an important part in the cleft formation seen at later ages. In relationship to the fluid content of the disc, it appears that the disc exhibits its greatest elasticity and power of resistance to pressure, towards the end of the 2nd and during the 3rd decade.

In reviewing the life history of the disc, it is worthy of note that the annulus fibrosus undergoes comparatively little change, while the nucleus pulposus exhibits continual structural changes from the time of its inception, throughout life. A consideration of these tissue changes, in relation to the clinical conditions of 'protrusion of the disc' or 'herniation of the nucleus pulposus', suggests that with gradual transformation of the disc into a uniform plate of fibrocartilage true herniation of the nucleus pulposus would become less frequent with advancing age.

Vascularization of the disc

In the literature, the accounts of the blood supply of the disc vary, and such variation depends on whether the cartilage plates are included in the designation 'disc' or not.

Keyes & Compère (1932) describe the adult disc as being avascular. Übermuth (1929, 1930) and Coventry *et al.* (1945) describe the vessels as being limited to the cartilage plates and as persisting up to 20 years of age. Luschka (1856) and Fick (1904) refer to vessels in the annulus only, and Brack (1929) describes vessels in the outer part of the annulus and blood islands as present in the cartilage plates in the earlier years of life. Böhmig (1930) gives an account of an elaborate blood supply furnished by two dorsal, ventral and axial vessels, and suggests that these supply the cartilage plates, annulus and nucleus. His illustrations certainly show the vessels in the cartilage plates, but they are apparently not present in the annulus and nucleus. The 'pinselartigen' branching which he describes for the disc vessels, are regarded by Schaffer (1910) as artefacts. Böhmig considers that these vessels persist till 25 years of age. Smith (1931) states that 'nutritive channels' are present in both annulus and nucleus of young, healthy discs. Those in the nucleus have a thin wall 'in which endothelial cells were not made out' but in the annulus, the channels, which could be traced into the cartilage plates, were lined with a single layer of flattened cells. Numerous leucocytes are present in the vessels in the annulus and nucleus, but red cells, absent in these two components, are present in the channels in the cartilage plates.

From the present investigation, it is concluded that, as far as the soft tissues are concerned, small blood vessels are present, running mainly concentrically among the lamellae in the postero-lateral aspects of the annulus, but that the nucleus pulposus remains avascular throughout life.

SUMMARY

1. Descriptions are given of the tissue changes taking place in the structural components of the human intervertebral disc, from birth to 80 years of age.

2. The disc is composed of a peripheral, firm annulus fibrosus comprising an outer zone of collagenous fibres, and an inner zone of fibrocartilage, and a central, soft nucleus pulposus. The fibre architecture of the annulus is intricate, and is present at birth. The nucleus pulposus, at birth, is formed mainly by modified notochordal tissue.

3. With the advance of age, the fibres in the annulus become coarsened and hyalinized, while in the nucleus, the notochordal element is replaced by fibrocartilage. Notochordal tissue appears to be absent after 10 years of age. The fibrocartilage of the nucleus, at first delicate and containing much fluid, becomes progressively coarsened and desiccated. Desiccation occurs in the disc as a whole, especially after 30 years of age, but is more marked in the nucleus than in the annulus. In advanced age, macroscopic distinction between nucleus and annulus is lost.

4. Transformation of the nucleus pulposus appears to result from addition to, and replacement of, the notochordal tissue by fibres and cartilage cells derived from the inner part of the fibrocartilaginous zone by a process of liquefaction.

5. The ultimate transformation of the intervertebral disc into a uniform plate of fibrocartilage, suggests that the incidence of true herniation of the nucleus pulposus would diminish with advancing age.

6. Small blood vessels are present, running concentrically among the lamellae in the postero-lateral regions of the annulus fibrosus, but the nucleus pulposus remains avascular throughout life.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Full-term intervertebral disc. Lumbar region. Median sagittal section showing notochordal area (*N.*), fibrocartilage zone (*F-c.*), zone of collagenous fibres (*C.F.*). Haematoxylin and eosin. × 8·4.

Fig. 2. Full-term intervertebral disc. Lumbar region. Sagittal section of periphery of annulus fibrosus showing crossing of fibres. Masson's trichrome. × 100.

Fig. 3. Full-term intervertebral disc. Lumbar region. Median sagittal section through notochordal area, showing remains of chorda reticulum and mucoid substance. Haematoxylin and eosin. × 135.

Fig. 4. Postnatal disc, 5 weeks. Lumbar region. Median sagittal section through nucleus pulposus and surrounding fibrocartilage, showing groups of notochordal cells (*N.*), mucoid substance in nucleus pulposus, liquefactive changes in surrounding fibrocartilage (*F-c.*). (Transitional areas.) Masson's trichrome. × 100.

Fig. 5. Postnatal disc, 4 years. Lumbar region. Median sagittal section of nucleus pulposus showing degenerated notochordal cells (*N.*) and delicate fibres. Masson's trichrome. × 100.

Fig. 6. Postnatal disc, 10 years. Lumbar region. Median sagittal section of nucleus pulposus showing groups of cartilage cells (*c.*) and mucoid substance (*M.S.*). Masson's trichrome. × 100.

PLATE 2

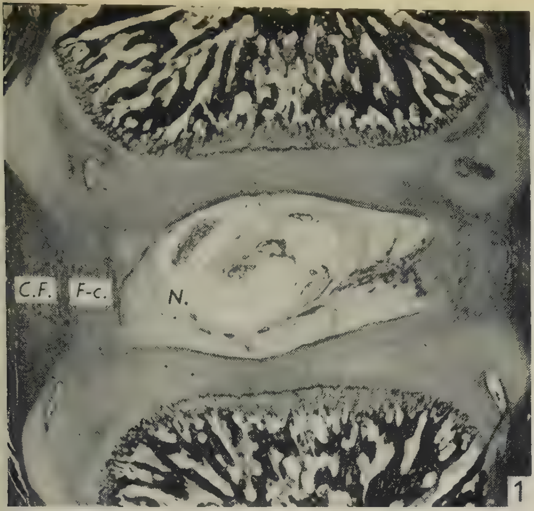
Fig. 7. Postnatal disc, 21 years. Lumbar region. Median sagittal section of nucleus pulposus showing loosely aggregated delicate fibrocartilage. Haematoxylin and eosin. × 80.

Fig. 8. Postnatal disc, 33 years. Lumbar region. Median sagittal section of nucleus pulposus showing fibrocartilage. Haematoxylin and eosin. × 80.

Fig. 9. Postnatal disc, 40 years. Lumbar region. Median sagittal section of nucleus pulposus showing fibrocartilage and nesting of cartilage cells. Masson's trichrome. × 100.

Fig. 10. Postnatal disc, 50 years. Lumbar region. Median sagittal section of nucleus pulposus showing cartilage cells with nests. Toluidin-blue. × 100.

Fig. 11. Postnatal disc, 64 years. Lumbar region. Median sagittal section of disc showing loss of clear demarcation between annulus and nucleus. Masson's trichrome. × 2·8.



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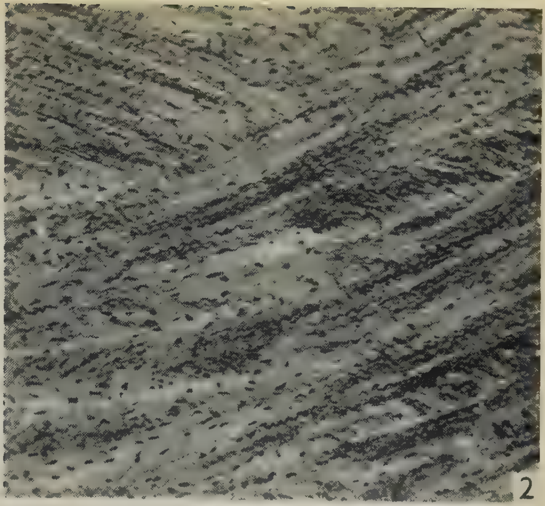
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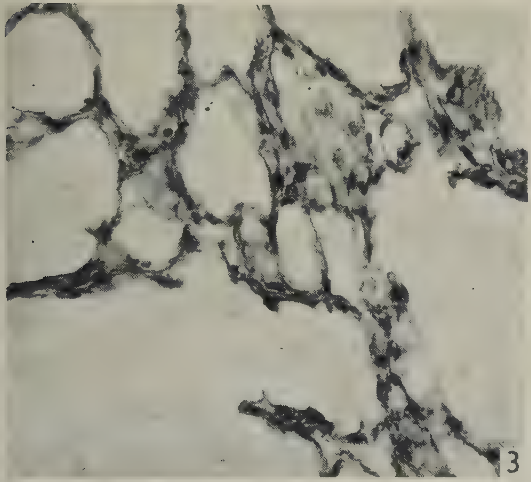
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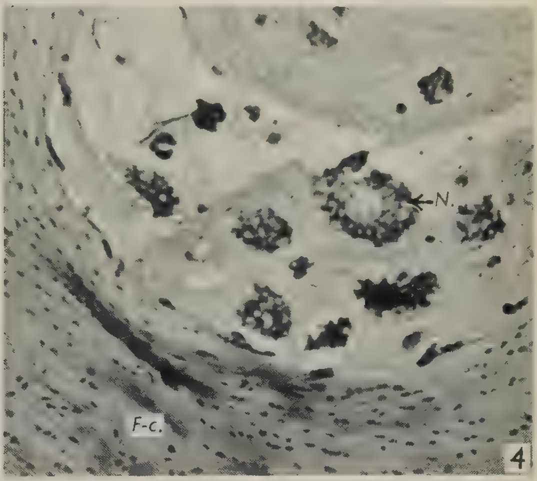

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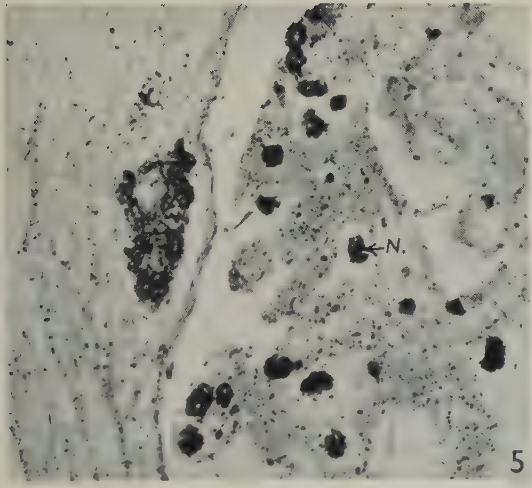

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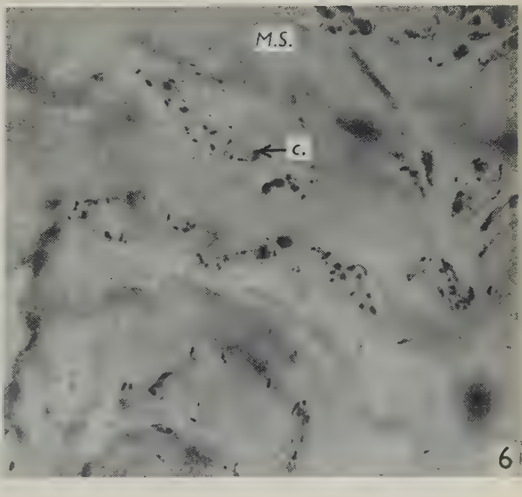
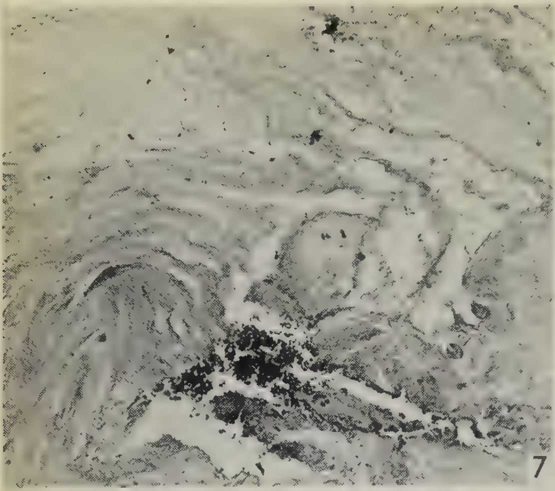


6

PEACOCK—OBSERVATIONS ON THE POSTNATAL STRUCTURE OF THE INTERVERTEBRAL DISC IN MAN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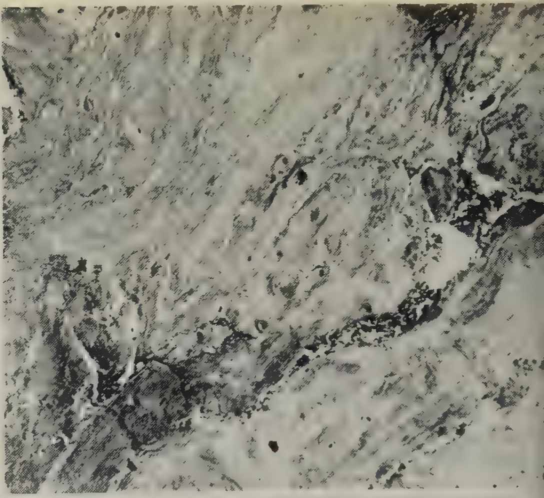

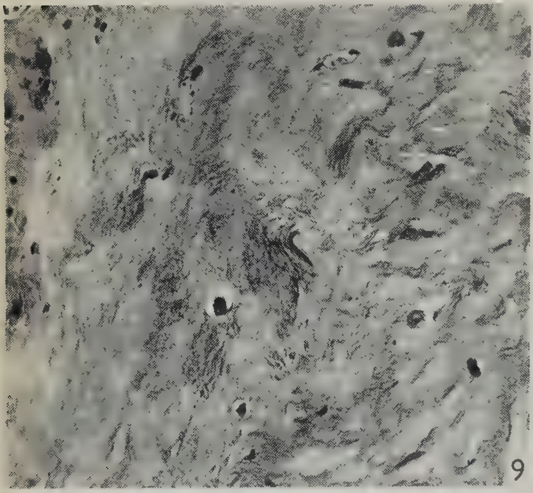
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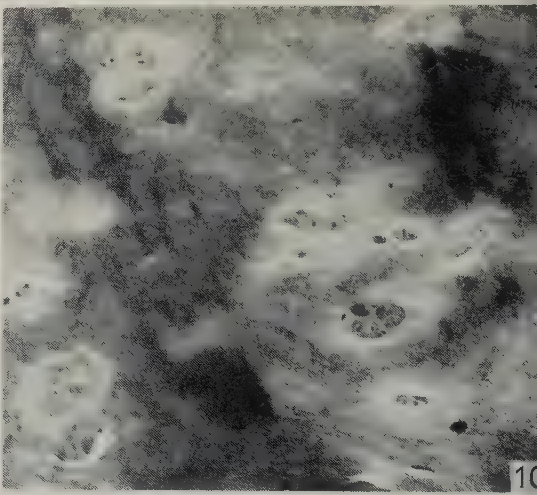
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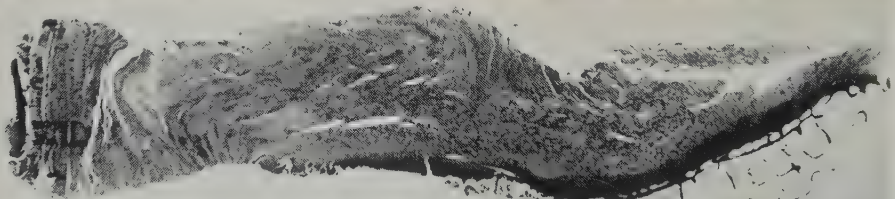
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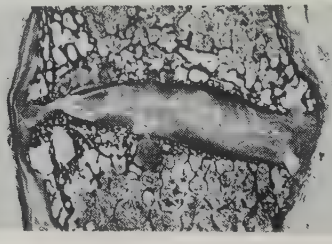
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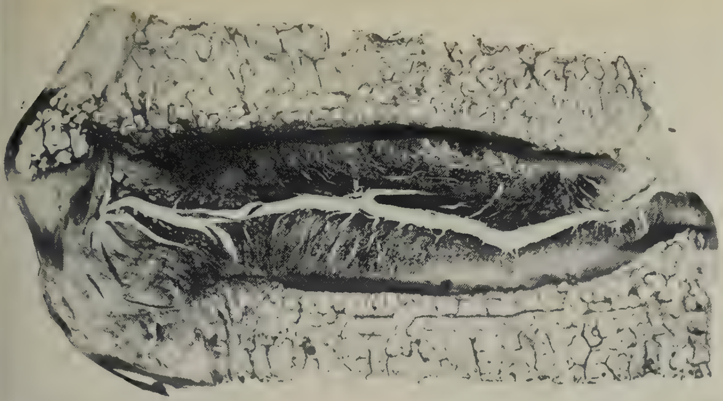
PEACOCK—OBSERVATIONS ON THE POSTNATAL STRUCTURE OF THE INTERVERTEBRAL DISC IN MAN



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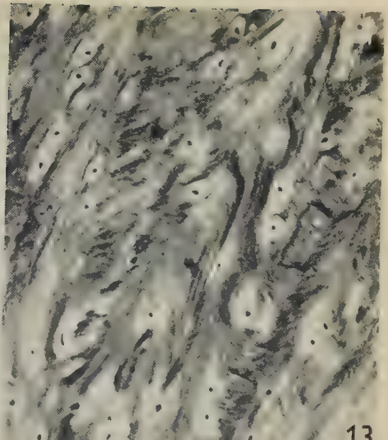

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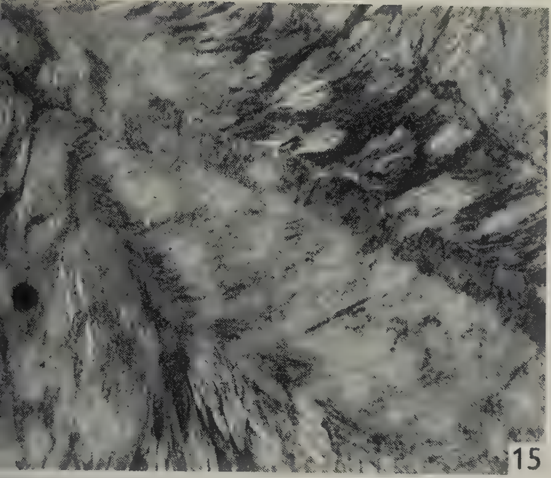

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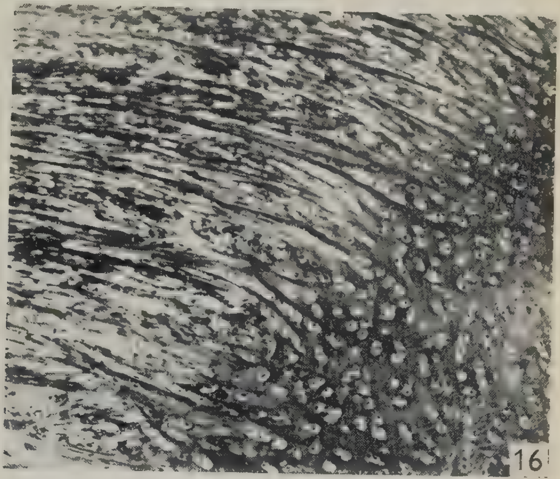

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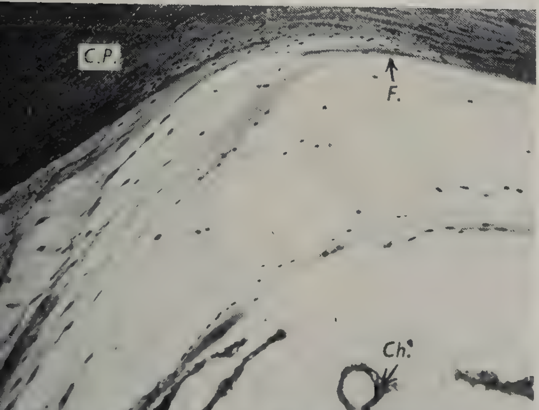

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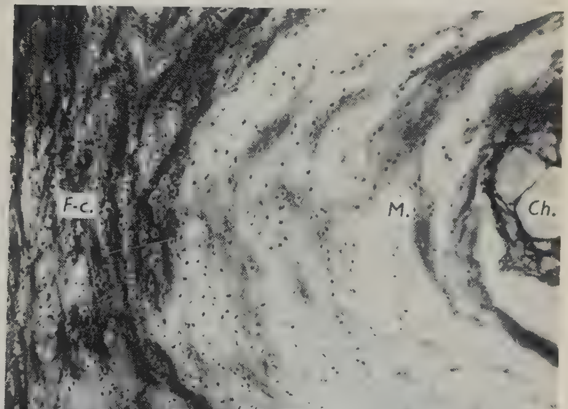

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EACOCK—Observations on the postnatal structure of the intervertebral disc in man

Fig. 12. Postnatal disc, 75 years. Lumbar region. Median sagittal section showing vertebrae and intervening disc and loss of demarcation between annulus and nucleus. Haematoxylin and eosin. ¾ natural size.

PLATE 3

Fig. 13. Postnatal disc, 75 years. Lumbar region. Median sagittal section of nucleus pulposus showing coarse fibrocartilage. Haematoxylin and eosin. × 180.

Fig. 14. Postnatal disc, 80 years. Lumbar region. Medial sagittal section showing loss of demarcation of annulus and nucleus and fissuring. Masson's trichrome. × 1·5.

Fig. 15. Postnatal disc, 80 years. Lumbar region. Medial sagittal section of nucleus pulposus showing indiscriminate arrangement and hyalinization of fibres, 'ghosts' of cartilage cells. Masson's trichrome. × 100.

Fig. 16. Full-term foetus. Lumbar disc. Median sagittal section of annulus fibrosus showing attachment of fibres of disc to cartilage plate. Masson's trichrome. × 73.

Fig. 17. Full-term foetus. Lumbar disc. Median sagittal section showing area of cartilage plate (*C.P.*) bounding nucleus superiorly, horizontal course of fibres (*F.*) in surface of plate turning down to join vertical component of annulus (transitional area). Chorda reticulum seen (*Ch.*). Masson's trichrome. × 73.

Fig. 18. Full-term foetus. Lumbar disc. Median sagittal section of disc showing transitional area between nucleus and annulus, notochordal reticulum (*Ch.*), separation of components of surrounding fibrocartilage (*F-c.*), mingling of the tissue components (*M.*). Masson's trichrome. × 73.

AN EXPERIMENTAL STUDY OF THE SECONDARY OLFACTORY CONNEXIONS IN *LACERTA VIRIDIS*

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INTRODUCTION

The part played by secondary olfactory connexions has long been considered of major importance in the evolution of the vertebrate forebrain. Recent experimental studies of these connexions, e.g. by Clark & Meyer (1947) and by Meyer & Allison (1949) have shown that their distribution is more limited, at least in mammals, than was generally supposed. In particular, it has been shown that few, if any, such fibres end in the septum or hippocampal formation. It was therefore considered to be of importance to re-investigate these connexions in the submammalian forebrain using experimental methods.

Lacerta viridis was chosen for these experiments, since specimens were easily obtained, and the normal anatomy of its forebrain has already been described in some detail by Goldby (1934). The method employed has been the examination of the results of the ablation of the olfactory bulbs and other lesions by a silver impregnation method in order to demonstrate axonal and terminal degeneration or the disappearance of fibres. Similar techniques were used very successfully by Armstrong (1950) in an investigation of the optic connexions in *L. vivipara.*

MATERIALS AND METHODS

Adult specimens of *L. viridis* of both sexes were used. The animals were kept in cages heated to about 30° C., both before and after operation.

Operative techniques

Under ether anaesthesia the skull was opened with a dental drill and the dura incised. Lesions in the brain were made by cutting with a cystotome. The cavity was filled with penicillin and sulphathiazole crystals, and the skull sealed with solution of Perspex in acetone which dried rapidly to form a very adequate substitute for the bone removed.

The lesions consisted in the removal of the main olfactory bulb either unilaterally or bilaterally, the removal of both the main and the accessory bulbs unilaterally, and division of the habenular commissure by cutting between the hemispheres in the appropriate position. Mortality was low after removal of the olfactory bulb or bulbs (2 in 29) but high where division of the habenular commissure was attempted (3 in 7). When it occurred, death appeared to be due to bleeding into the cranial cavity. Sepsis occurred in two experimental animals but seemed to cause them no

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inconvenience, being discovered only when the prepared sections were examined; these two brains have been excluded from this study.

The animals were killed by decapitation while under ether anaesthesia, the brains exposed as rapidly as possible and fixed *in situ* for 24 hr. before removal from the skull.

Histological methods

(i) Four Nissl series were prepared from normal specimens. The brains were fixed in formol-acetic-alcohol; two transverse series were stained with gallocyanin and one horizontal and one sagittal series with cresyl violet. The sections were all cut at 10μ after paraffin embedding.

(ii) Silver impregnated series were prepared by Nonidez's (1939) technique. All sections were cut at 5–8μ. The normal material consisted of two transverse and one horizontal series. The experimental material consisted of twenty-seven brains from which satisfactory series were prepared and in which the lesion, survival time, etc. were as follows:

(*a*) Unilateral removal of the main olfactory bulb; three transverse series with survival times of 2, 10 and 14 days, and ten horizontal series with survival times of 36, 51 and 54 hr. and 3, 4, 5, 7, 10, 11 and 120 days.

(*b*) Unilateral removal of both main and accessory olfactory bulbs; three transverse series with survival times of 2, 7 and 14 days, and eight horizontal series with survival times of 48, 51, 54 hr. and 3, 4, 5, 9 and 14 days.

(*c*) Bilateral removal of the main olfactory bulb; one transverse series with survival time of 13 days.

(*d*) Division of habenular commissure; two transverse series with survival times of 3 and 9 days.

OLFACTORY AND RELATED SYSTEMS IN NORMAL MATERIAL

The anatomy of the olfactory and related systems in the forebrain of *L. viridis* has already been described by Goldby (1934) on the basis of normal material. Further examination of similar material has added little to his findings, and it is necessary here to refer only to certain of the fibre tracts which are particularly important for the experimental work to be reported.

In addition to the well-known lateral olfactory tract, intermediate and medial olfactory tracts have also been described. The latter are not well defined nor very clearly separable from each other. They consist of fibres related to the surface of the olfactory tubercle ventrally and the septum medially. Those fibres lying superficially in the olfactory tubercle form the intermediate olfactory tract. They are closely related to the fibres of the forebrain bundles (from which they are not clearly distinguishable) and, medially, are virtually continuous with the ventral part of the medial olfactory tract. This medial olfactory tract can be traced into the dorsomedial and medial parts of the anterior olfactory nucleus, through which the fibres appear to be continued into the septum and the anterior part of the hippocampal cortex. For convenience of description it will be divided into dorsal and ventral parts, although the distinction is not marked by any clear boundary in the normal brain. The dorsal part includes those fibres which appear to pass into the anterior hippocampal cortex; the ventral part passes caudally on the superficial aspect of the ventral part of the septum and in close association with the intermediate tract. As the fibres of both the intermediate olfactory tract and the ventral part of the medial olfactory tract are followed caudally they become closely related, not only to the fibres of the forebrain bundles but also to 'tuberculo-septal' fibres, the anterior olfacto-habenular tract and, more posteriorly, the diagonal band system. Olfactory tract fibres might contribute to any of these tracts or systems of fibres, but whether they do, and if so, to what extent, cannot be determined from normal material.

The term 'diagonal band' is applied to fibres which cross the base of the hemisphere obliquely on its ventral surface immediately behind the olfactory tubercle. They may be seen in all planes of section but most clearly in horizontal sections which cut the region tangentially (Pl. 2, figs. 8–10). They are so named from an apparent similarity to a band of fibres in this situation described by Broca (1879) in mammals. On the medial side, they may be traced dorsally through the nucleus of the diagonal band and, perhaps after a relay, to the superficial aspect of the hippocampal cortex; in this medial part of their course they are sometimes referred to as the 'olfactory radiations of Zuckerkandl'. Laterally they may be traced into the superficial part of the amygdaloid complex where they are accompanied by fibres of the more deeply situated amygdalo-pre-optic tract (Goldby, 1934), and the superficially placed lateral cortico-habenular tract, from which they are practically indistinguishable. Some fibres of the diagonal band system may enter the stria medullaris lateralis and reach the habenular region. In their course medio-laterally on the base of the brain they are closely related to the pre-optic and supra-optic nuclei with which they may also be connected.

A compact bundle readily identified in the septum (Pl. 1, fig. 1) is the anterior olfacto-habenular tract (Goldby, 1934, and others). It is described as beginning in the dorsal part of the anterior olfactory nucleus whence it can be followed caudally and ventrally through the septum to pass through the diagonal band to the stria medullaris lateralis and the habenular region. Despite its name, its precise origin and termination and the direction in which it conducts are unknown. Together with the amygdalo-pre-optic connexion referred to above, it might well be included in the diagonal band system, and it is clear that a satisfactory analysis of the fibres which cross the ventral surface of the hemisphere in the region of the diagonal band cannot be made in normal material.

The stria medullaris is a bundle of fibres lying along the dorso-medial border of the thalamus, closely related to the habenular nuclei and contributing many fibres to the habenular commissure. In *L. viridis* it may be divided into medial and lateral parts.

The medial part is formed from the medial cortico- and olfacto-habenular tracts. The former is said to derive crossed and uncrossed fibres from the hippocampal cortex, the latter to be connected with the hypothalamic and pre-optic region (Goldby, 1934). This description is tentative since the direction of conduction in these fibres is not known.

The lateral part, the 'stria medullaris lateralis', is of greater interest from the point of view of this investigation. It appears to receive fibres from the amygdaloid region and pyriform cortex through the lateral cortico-habenular tract and also the fibres already referred to as the anterior olfacto-habenular tract. A third component, the lateral olfacto-habenular tract, is described as joining it from the pre-optic region. The stria medullaris lateralis passes dorsally from the caudal end of the base of the hemisphere lateral to the lateral forebrain bundle, and in the angle between this bundle and the anterior margin of the optic tract (Pl. 2, fig. 7). Dorsal to the forebrain bundles it joins the medial part of the stria medullaris to turn caudally and run back to the habenular commissure. It is clear that the very close relation between the origin of the stria medullaris lateralis and the diagonal band system would make an extensive connexion between the two possible, although contributions to the stria medullaris from the anterior olfacto-habenular tract and the lateral cortico-habenular tract only have so far been described.

THE NATURE OF DEGENERATIVE CHANGES IN THE OLFACTORY SYSTEM OF *LACERTA VIRIDIS*

In *L. vivipara*, Armstrong (1950) found that the maximal changes in optic tract fibres were present about 14 days after the optic nerve had been cut. These changes took the form of irregular varicosities along the whole length of the fibres and of rings and solid bulbs on what were taken to be their endings. In the olfactory system of *L. viridis* few, if any, changes of this kind were seen in animals killed more than 3 days after operation. The only change found consistently in all specimens killed at such times was a loss of fibres from situations which varied according to the nature of the lesion. Sometimes some yellowish debris was found in a region from which fibres had disappeared, but this was not constant nor conspicuous enough to serve as a useful criterion of degeneration. The disappearance of fibres is apparently complete at a very early stage. After removal of the main or of the main and accessory olfactory bulbs, the loss of fibres was obvious in animals killed 3 days after the operation. In specimens killed after 5 days the loss was perhaps slightly more marked, but no evidence for any further loss could be found in specimens killed after longer intervals.

In all, ten animals were killed between 36 and 72 hr. after operations on the main or main and accessory olfactory bulbs and examined for evidence of axonal or terminal degeneration. In four of these (36, 51 and two 72 hr. specimens) the results were negative, although in the 72 hr. specimens many fibres had undoubtedly been lost. In two, 48 and 54 hr. specimens respectively, axonal changes in the form of irregular varicosities were present in the olfactory tracts; in the former, end bulbs and rings appeared also to be present over the olfactory tubercle (Pl. 3, figs. 12, 13 and 15) and pyriform cortex. The remaining four specimens showed only scanty evidence of axonal changes, and in no case throughout all the systems which were thought likely to be affected. Fusiform swellings along the course of fibres or, more commonly, near or at their apparent terminations again formed the principal evidence. The anatomical localization of these changes will be described in a later section. Here only their general nature and the question of their reliability as evidence will be considered.

An observation of the loss of fibres was taken as satisfactory evidence that the fibres in question had been cut by the lesion, or their cells of origin removed. provided that it could be made consistently in all other specimens with similar lesions. The ease with which a loss of fibres can be seen naturally varies with its degree. Where it occurs consistently and completely in a compact and virtually homogenous tract, no difficulty arises; if, however, the loss occurs in a compound fibre system and is incomplete, its recognition may be much more difficult, and the loss of only a few fibres can pass unnoticed. Nevertheless, a thinning out of fibres can be satisfactory evidence when observed unequivocally in a number of specimens all with similar lesions and if carefully controlled by comparison with normal specimens. It is clear, of course, that such observations can give no information about the actual termination of fibres.

In these experiments the evidence obtained from the positive observation of degenerative changes in axons or their endings was, by itself, inadequate for comprehensive anatomical interpretation. The changes were variable and inconstant in their appearance in different specimens. That they were artefacts due to fixation, or some other technical factor, may be excluded, since they were never seen in normal specimens nor in any of the specimens killed more than 3 days after operation (except that a very few such changes were sometimes seen up to 5 days after operation). Moreover, the situations in which they were found, though not usually so widespread as would have been expected, were always regions in which olfactory tract fibres appeared to be present in normal preparations or from which fibres had disappeared in specimens kept for a longer time after removal of the olfactory bulb. One may conclude that although axonal and probably terminal degenerative changes occur after injury to fibres of the olfactory system, they are demonstrable only for a short time during the first 3 days. The variability, even in animals killed at this particular time, probably means that degenerative changes do not become demonstrable at the same moment in all the fibres affected by the lesion. At any one time only a few fibres may be in a condition in which the changes can be demonstrated, at least by the Nonidez technique, and they do not appear to remain in this condition for long. It is also probable that degeneration does not proceed at exactly the same rate in different specimens.

For these reasons, the anatomical findings to be reported are based primarily on the observation of a loss of fibres 3 or more days after removal of the olfactory bulbs, or other lesion, while the degeneration of axons (and possibly terminals) in specimens killed after shorter intervals has provided useful confirmatory evidence. As already indicated the findings in the latter, though less complete, were in no case inconsistent with the findings in the specimens kept for longer periods.

EXPERIMENTAL RESULTS

Some of the observations made after removal of the main or of the main and accessory olfactory bulbs merely confirm what was already reasonably well established on the basis of previous work, chiefly in normal material, and these can be dealt with briefly.

Removal of the main bulb (nine specimens) led, after 3 or more days, to a substantial but incomplete loss of fibres from the olfactory peduncle, and a similar loss from among the cells in the dorso-lateral part of the anterior olfactory nucleus. Behind this nucleus the lateral olfactory tract showed a slight thinning of its fibres, but only in the anterior part of its course where it runs on the surface of the brain along the lower border of the pyriform cortical area. Posteriorly, where it dips beneath the surface into the amygdaloid complex, no difference from the normal side could be seen. There was also some loss of fibres from the superficial aspect of the anterior part of the pyriform cortex.

When, in addition to the main bulb, the accessory bulb was removed (seven specimens) and the animal allowed to survive 3 days or more, the results, so far as the above structures are concerned, were very similar though more marked. There was complete loss of fibres in the stump of the olfactory peduncle and the loss of fibres in the lateral olfactory tract, though still incomplete, could be followed throughout its length into the amygdaloid complex, including the hypopallium posterior.

The observations based on the unilateral removal of both main and accessory bulbs received some confirmation from two specimens killed less than 3 days after operation. In one (L.V. 52, 48 hr. survival), varicosities, some of them vesicular, were seen on nerve fibres throughout the superficial parts of the anterior olfactory nucleus, over the anterior half of two-thirds of the pyriform cortex, throughout the length of the lateral olfactory tract, in the hypopallium posterior and over the surface of the olfactory tubercle (Pl. 3, figs. 12, 13 and 15). In another (L.V. 69, 54 hr. survival) larger varicosities were present throughout the length of the lateral olfactory tract and in the medial olfactory tract (Pl. 3, fig. 14), but not over the pyriform cortex. In two further specimens (L.V. 62 and 70) killed 51 and 54 hr. respectively after removal of the main bulb only, the results, so far as the anterior olfactory nucleus, pyriform cortex and lateral olfactory tract were concerned, were entirely negative.

One may base the following conclusions on these experiments. The fibres in the peduncle are wholly efferent from the olfactory bulbs, and many of them pass no farther than the anterior olfactory nucleus. A proportion of fibres from the main bulb pass without relay into the lateral olfactory tract and probably end in the anterior part of the pyriform cortex; none, or very few, reach the amygdala. Fibres from the accessory bulb, however, pass without relay through the lateral olfactory tract as far as the amygdaloid complex, although it is not possible to say that all do so. Some may end in other regions, e.g. in the pyriform cortex, but there is no evidence for or against this possibility. It is also possible, of course, that fibres from the main bulb influence the amygdala through the lateral olfactory tract after relay in the anterior olfactory nucleus.

In addition, a loss of fibres superficial to the olfactory tubercle occurred in all the series where bulbar ablation was carried out 3 or more days before the animal was killed. In one with a survival period of 54 hr. (L.V. 69) large axonal varicosities were seen in the ventral part of the medial olfactory tract (Pl. 3, fig. 14) and all over the surface of the olfactory tubercle, and in one with a survival time of 48 hr. (L.V. 52) small, solid and vesicular structures were found throughout the superficial part of the tubercle (Pl. 3, figs. 12, 13 and 15). These findings confirm the commonly held view that secondary olfactory fibres end over the surface of the olfactory tubercle.

Certain negative results may also be reported briefly. None of the specimens showed any change whatever in the fibres among the cells of the septal nuclei nor in fibres related to the hippocampal and dorsal cortical areas. If these structures receive any direct bulbar connexions they must consist of very few fibres indeed, and the fibres which appear to reach them from the dorsal part of the medial olfactory tract probably arise in the anterior olfactory nucleus. Furthermore, bulbar ablations, whether affecting the main bulb only or both main and accessory bulbs, led to no detectable evidence of degeneration in any component of the anterior commissure either as a loss of fibres or as degenerative axonal changes.

The effects of removal of the olfactory bulbs on the ventral part of the medial olfactory tract were more striking and extended more widely than was expected, and must be described at greater length. They were produced by removal of the main bulb only, and were not increased when the accessory bulb was removed as well, so that all the fibres to be considered must originate in the main bulb. One specimen (L.V. 34) in which one main olfactory bulb was removed 3 days before the animal was killed may be taken as typical. The silver impregnation was particularly good and, in spite of the short survival time, the fibre loss was quite definite.

On the side of the operation there was an obvious disappearance of fibres from the region of the ventral part or the medial olfactory tract along the ventro-medial border of the hemisphere. This was continuous caudally with a considerable and unexpected loss of fibres from the diagonal band system caudal to the olfactory tubercle (Pl. 2, fig. 10).

Equally unexpected and more striking was the virtually complete disappearance of the anterior olfacto-habenular tract from the opposite (unoperated) hemisphere (Pl. 2, fig. 9). This, as has been mentioned, is a small but well-defined and particularly compact tract, especially about the middle part of its course, where it lies superficially in the septum (Pl. 1, figs. 1–4).

Careful examination of the stria medullaris lateralis (through which the fibres of the anterior olfacto-habenular tract have been said to run to the habenula) showed, on the unoperated side, a very marked but not quite complete loss of fibres from a small area situated caudally in the bundle adjacent to the anterior margin of the optic tract (Pl. 2, fig. 5). On the operated side this area was occupied by apparently normal fibres which filled the concavity of a crescentic area from which fibre loss was very marked but, again, not complete (Pl. 2, fig. 6). In other words, the appearance in the stria medullaris lateralis on the right and left sides was such that one appeared as the reverse or 'negative' of the other (Pl. 2, figs. 5, 6) and both differed from the normal (Pl. 2, fig. 7).

A very similar difference between the two sides was seen in horizontal sections cutting the diagonal band tangentially on the ventral aspect of the hemisphere (Pl. 2, figs. 9, 10). In fig. 10, the operated side, the anterior olfacto-habenular tract can be seen as a compact, deeply impregnated bundle of fibres postero-medial to the main part of the diagonal band in which fibres are fewer than normal. The corresponding region on the unoperated side (fig. 9) is almost free of fibres, while on this side the main part of the diagonal band contains its normal complement.

The appearances described were seen throughout the stria medullaris lateralis, though with rather more difficulty in the posterior part of its course where it runs along the dorso-medial border of the thalamus. In the habenular commissure an appearance in which bundles of fibres were seen to interdigitate with strips from which fibre loss was almost complete, replaced the normal uniform appearance of closely packed fibres. In no case was any loss of fibres seen in the plexus surrounding the cells of the habenular nuclei.

It is clear that these regions in which a loss of fibres was observed form a continuous tract passing caudally as the ventral part of the medial olfactory tract to the diagonal band and stria medullaris lateralis on one side, across in the habenular commissure, and then rostrally on the opposite side in the stria medullaris lateralis and anterior olfacto-habenular tract; and that the fibres which disappear are secondary olfactory fibres from the main olfactory bulb which end at or near the anterior extremity of the so-called anterior olfacto-habenular tract of the opposite side, that is, in the dorsal part of the anterior olfactory nucleus and perhaps in adjacent parts of the cortex.

The evidence for the existence of such a long and circuitous crossed secondary olfactory tract, not previously described, needs careful scrutiny. If the findings in L.V. 34 are to be accepted at their face value there does not appear to be any other interpretation than the one which has been given; but conclusions based on observations of a relative or even a complete loss of fibres are always open to the criticism that the apparent loss may be the results of a fortuitous deficiency in the silver impregnation in certain situations. Two things, however, seem to make this criticism inapplicable.

First, although the impregnation in the quite large number of specimens prepared varied somewhat, the variations did not take the form of patchy deficiencies scattered widely in the forebrain. Where the impregnation was deficient a whole region was affected, and all the fibres in it; this defect was most common in the extreme anterior end of the hemisphere, affecting the olfactory peduncle and the anterior olfactory nucleus but did not affect the regions in which the diagonal band and stria medullaris are situated.

Secondly, it was found that the observations which have been described for L.V. 34 could be made in all the specimens which had been subjected to a similar operation. Sixteen specimens were prepared in which the main or main and accessory olfactory bulbs were removed unilaterally and in which the survival time was 3 days or longer. In all of these, the absence of the anterior olfacto-habenular tract on the unoperated side was unequivocal; and in all the tract was present on the operated side. Sometimes a little yellowish debris was found in the position of the tract on the unoperated side (e.g. in L.V. 34) but, in all, the contrast with the normal tract was definite. It may be pointed out that the position of these tracts in the septum is such as to make the comparison of the two sides particularly easy, and that they were clearly present bilaterally in three normal specimens, and in three others, not referred to elsewhere in this paper, with lesions which did not affect the olfactory bulbs or tracts. The loss of fibres from the region of the diagonal band was almost equally clear, especially in the horizontal series. In the stria medullaris lateralis the changes observed are not quite so striking. In the specimen chosen to illustrate these changes the impregnation was good and the fibres well defined against a clear background; some other specimens gave equally clear results, and in the remainder careful examination always established that similar changes had occurred. With the ventral part of the medial olfactory tract the position was somewhat similar, but here the area of the loss of fibres was less clearly defined owing to intermingling with adjacent fibres, e.g. of the medial forebrain bundle. Nevertheless, the loss of fibres was consistently observed, although more obvious in some specimens than in others. In the habenular commissure excessive impregnation rendered the recognition of areas of fibre loss impossible in two series, but in the others it was observed consistently and took the form already described in L.V. 34.

Attempts were made to obtain further evidence from the presence of degenerative changes in axons at short periods after operation. Eight animals were killed at intervals varying between 36 and 54 hr. after unilateral bulbar ablation. In one of these (L.V. 69), killed 54 hr. after operation, large axonal varicosities were found in the olfactory tracts; those in the ventral part of the medial olfactory tract (Pl. 3, fig. 14) could be traced through the diagonal band and the stria medullaris to the habenular commissure and then forward through the opposite stria into the anterior olfacto-habenular tract. In three others, killed at or about 48 hr. after operation, varicosities were present on the axons of the opposite anterior olfacto-habenular tract near its termination in the dorsal part of the anterior pole of the opposite hemisphere but not elsewhere. The other four specimens gave negative results as far as this system is concerned, and it seems reasonable to assume that this was due either to a failure in the technique or to the absence of a sufficient number of fibres with demonstrable changes at the particular time when the animal was killed. It may be added that in none of these short-term specimens was any evidence of degenerative change seen within the habenular nuclei.

While giving support to the conclusions based on observations of the disappearance of fibres, the number of specimens showing positive degenerative changes was not large, and the following additional tests were carried out.

In one specimen, the main olfactory bulbs were removed bilaterally. After a survival period of 13 days it was found that there was a complete and unequivocal disappearance of the anterior olfacto-habenular tract on both sides. This series was cut transversely and the loss was particularly obvious in the septal region where the tract is compact and isolated from other fibre systems. The ventral part of the medial olfactory tract had also disappeared bilaterally, while the region of the diagonal band caudal to the olfactory tubercle was very conspicuously poor in fibres, the caudal continuation of the ventral part of the medial olfactory tract and the closely related anterior olfacto-habenular tract having disappeared from this situation. The so-called diagonal band now consisted only of fibres in the medial and ventro-lateral walls of the hemisphere, separated from one another ventrally by an area virtually free of fibres. The stria medullaris lateralis consisted only of a few fibres set in a pale-staining matrix, and its appearance throughout resembled that seen only in localized parts after unilateral bulbar ablation. In the habenular commissure the loss of fibres, as compared with the normal, was most striking.

In two specimens the habenular commissure was divided successfully. In one (L.V. 60), 9 days survival, the sections showed that the commissure had been divided completely, and that the knife had passed laterally into the thalamus between the nucleus rotundus and the lateral geniculate body, also damaging the stria medullaris on that side. So far as could be seen, no damage had been caused to the nucleus rotundus itself nor to the lateral forebrain bundle. There was a little damage to the hippocampal cortex of one hemisphere, and some loss of the underlying alveus fibres was observed. Apart from these direct effects of the lesion the only change seen in the hemispheres was the complete bilateral absence of the anterior olfacto-habenular tract, while fibre loss had occurred bilaterally in a localized area within the stria medullaris lateralis, corresponding to that area from which fibres were lost on the unoperated side after unilateral bulbar ablation. In the second specimen (L.V. 47), survival period 3 days, the lesion appeared to be limited to the habenular commissure and the posterior commissure immediately behind it. Large axonal varicosities were found bilaterally in the stria medullaris lateralis and in the whole extent of the anterior olfacto-habenular tract affecting the majority, if not all, of its fibres (Pl. 3, fig. 11). Smaller varicosities were also seen bilaterally in the lateral cortico-habenular tract, though affecting a smaller proportion of its fibres; no other changes were observed within the hemispheres.

These two experiments were successful within their limits, in that they demonstrate that the fibres of the anterior olfacto-habenular tract conduct rostrally and not caudally as had been thought and as their name implies, and also that they cross in the habenular commissure. They cannot, of course, confirm the bulbar origin of these fibres, but taken with the results of the bulbar ablation experiments they leave little doubt of the existence of a crossing system of secondary olfactory fibres which, having crossed, form the anterior olfacto-habenular tract.

The fibres of the diagonal band, where they are found in the posterior part of the septum in close relation to the nucleus of the diagonal band, were not affected by any of the operations performed. The elimination of the crossing secondary olfactory fibres from the caudal end of the base of the hemisphere after unilateral or, better, bilateral bulbar ablation made the investigation of these fibres much easier and at the same time widely interrupted the apparent continuity of the whole system between medial and lateral centres of the hemisphere. As the fibres in the septum are traced ventrally it can be seen that they do not pass laterally across the base of the hemisphere behind the olfactory tubercle; they pass to the region of the supra-optic nucleus where they appear to end. Virtually all the fibres running obliquely across the base of the hemisphere caudal to the olfactory tubercle, i.e. in the most ventral part of the 'diagonal band', are secondary olfactory fibres—the crossed fibres of the anterior olfacto-habenular tract medially and the uncrossed fibres of the ventral part of the medial olfactory tract on their lateral aspect.

The fact that the medial fibres of the diagonal band end in or near the supra-optic nucleus can be confirmed by an examination of normal material, but is by no means as clear as when the secondary olfactory fibres are eliminated.

DISCUSSION

As already stated, the conclusions which concern the lateral olfactory tract differ only slightly from those reached in earlier accounts. Goldby's (1937) observations in Marchi material indicated that secondary olfactory fibres from the main bulb ended in the anterior olfactory nucleus, and it appears from the present work that some also reach the anterior parts of the pyriform cortex. Structures possibly representing degenerating terminals were found in one experimental series relevant to this question. None was seen in actual contact with the body of a cell, so the relationship may be predominantly axo-dendritic rather than axo-somatic, but the evidence is inadequate for a more definite statement.

The intermediate olfactory tract appears to originate largely in the main olfactory bulb, but the termination of its fibres over the olfactory tubercle had already been inferred from normal material and confirmed experimentally (Goldby, 1937). The condition in mammals is similar, since degeneration has also been found in the lateral and ventral parts of the olfactory tubercle in the rabbit (Clark & Meyer, 1947) and in the anterior and lateral parts of the tubercle in the monkey (Meyer & Allison, 1949) after bulbar ablation.

The dorsal part of the medial olfactory tract has been shown to originate at least mainly in the accessory bulb and to have a less extensive distribution than usually supposed. No evidence was found that any of these fibres reach the septum and the hippocampal formation. They appear to end in the dorsal and medial parts of the anterior olfactory nucleus. At the same time it is impossible to deny that a few may reach the extreme anterior and least differentiated part of the hippocampal cortex where it blends with the anterior olfactory nucleus. This lack of any substantial secondary olfactory connexion with the hippocampal formation is in accord with the experimental findings of Clark & Meyer in the rabbit and with those of Meyer & Allison in the monkey.

Bulbar ablation caused no loss or degeneration of fibres in the 'olfactory component' or indeed in any part of the anterior commissure or in the opposite olfactory bulbs; Goldby (1934) was of the opinion that at least the majority of fibres in the 'olfactory component' of the anterior commissure arose in the anterior olfactory nucleus and adjacent structures. These findings in *L. viridis* differ from those of Clark & Meyer in the rabbit where, after bulbar ablation, degeneration was observed in the anterior commissure, in the opposite olfactory bulb and in the bed nucleus of the opposite stria terminalis and central amygdaloid nucleus. It is true that the degeneration was found to be more extensive when the lesion involved the anterior olfactory nucleus, but fibres of bulbar origin were believed to cross in the anterior commissure.

The most surprising findings are those interpreted as demonstrating the presence of an olfactory tract which takes a circuitous route through the habenular commissure to end near the anterior pole of the opposite hemisphere. The different parts of this tract have all been described in normal material, but never as a continuous system of fibres. Various names have been applied to these parts, that of one, the anterior olfacto-habenular tract, being based on its presumed connexions, those of others, e.g. the stria medullaris lateralis and diagonal band, simply defining their topographical position. Although in themselves non-committal, these latter terms have often, as a result of usage, come to imply specific connexions, e.g. the diagonal band, which is generally understood to consist of fibres linking lateral olfactory centres with the septum and hippocampal formation.

It is clear, therefore, that if the conditions found in *L. viridis* are generally present in other reptiles, a radical revision of terminology is desirable. This is particularly obvious in the case of the anterior olfacto-habenular tract, which, of course, becomes a secondary olfactory tract running rostrally after crossing in the habenular commissure; it is doubtful if it has any functional connexion with the habenular nuclei, either through axonal or collateral endings. It is hardly less obvious in the case of the 'diagonal band', which needs some further discussion since its presumed connexions, linking medial and lateral olfactory centres, have been used by Johnston (1915) as a basis for theoretical conclusions concerning the evolution of the temporal pole and the general pallium.

In *L. viridis* and probably in other reptiles, fibres in the position of the diagonal band and connecting medial with lateral centres in the hemisphere, if present at all, are extremely few. Most, if not all, of the fibres which cross the base of the hemisphere obliquely behind the olfactory tubercle belong to the secondary olfactory system which has been described, some passing caudally, others rostrally. The medial part of the diagonal band system, described as ascending in the septum to the hippocampal cortex, and closely related to the nucleus of the diagonal band, does not cross the base of the hemisphere and, therefore, does not reach the lateral olfactory centres. In specimens in which removal of the olfactory bulbs resulted in the disappearance of the secondary olfactory fibres, it may be seen that these fibres end in or very close to the supra-optic nucleus. It appears that the nucleus of the diagonal band should be associated with the supra-optic nucleus rather than with the olfactory system and that the alternative name of these fibres, the 'olfactory radiations of Zuckerkandl', is also unsatisfactory. At the lateral end of the diagonal band system fibres also persist after bulbar ablations. Again, in such specimens, it is easy to see that these fibres are not continuous across the base of the hemisphere with those in the septum. They originate or end laterally and appear to join the stria medullaris lateralis, probably representing the lateral cortico-habenular tract described by previous authors. In brief, what has been described as the diagonal band in *L. viridis* should be split into three independent parts, namely:

(1) Fibres situated medially among the cells of the nucleus of the diagonal band and extending dorsally to the hippocampal cortex and ventrally to the region of the supra-optic nucleus.

(2) Fibres lying ventrally behind the olfactory tubercle and forming crossed and uncrossed parts of a secondary olfactory system.

(3) Fibres lying laterally over the surface of the amygdala and probably forming the lateral cortico-habenular tract.

A study of the published descriptions of the reptilian forebrain does not show anything which is necessarily inconsistent with the suggestion that a crossed secondary olfactory tract, similar to the one found in *L. viridis*, is present in other species. There is very little which would justify a more positive statement. In *Cistudo* Johnston (1915) described fibres of the medial olfactory tract as passing caudally to join his 'diagonal band', and Crosby (1917), in the alligator, found similar fibres which passed caudally into close relation with the nucleus of the diagonal band. In *Tupinambis nigropunctatus* Curwen (1937) has described a tractus olfacto-hypothalamicus superficialis, which in her figures occupies a position very similar to the ventral part of the medial olfactory tract in *Lacerta*. She describes it as passing caudally and laterally in close association with the anterior olfacto-habenular tract, and states that it joins the stria medullaris and that some of its fibres may reach the habenular nuclei; most, she thinks, join the medial forebrain bundle from the stria medullaris. Curwen states that this tract arises in the superficial part of the olfactory tubercle; since her material consisted of a single transverse series stained by the Weigert method, one cannot feel that the evidence for this is adequate, and the fact that the fibres could be traced to the stria medullaris along a course practically identical with that of the ventral part of the medial olfactory tract in *Lacerta* suggests strongly that they belong to the same tract. There are, however, other differences in her description, such as the large contribution from the stria medullaris to the medial forebrain bundle, and in the absence of further evidence, preferably from experimental material, one can say no more than that Curwen's findings in *Tupinambis* suggest strongly a close resemblance to the arrangement of the olfactory tracts found in *Lacerta*, and establish a case for further investigation. It may be added that she also describes an anterior olfacto-habenular tract which agrees in all essential particulars with other descriptions in normal material (e.g. Goldby, 1934), and also sends some of its fibres into the habenular commissure. The corresponding tract described by de Lange (1911) as 'the olfacto-habenular tract' was thought to cross completely in this commissure.

While observations such as these suggest that the condition found in *Lacerta* may well be general throughout Reptilia, it must also be admitted that a continuous bundle of fibres passing back as part of the medial olfactory tract to enter the stria medullaris and cross in the habenular commissure to form the 'anterior olfacto-habenular' tract of the opposite hemisphere, has never previously been described. From the nature of this bundle it is extremely unlikely that any but experimental methods will reveal its presence, and until these methods have been applied more extensively in studies of the reptilian brain, it would be premature to suggest altering a well-established terminology.

A wider survey of the literature of the vertebrate forebrain shows that tracts, which might form part of a similar bundle of secondary olfactory fibres, exist in other orders. In Amphibia, the ventral division of the anterior olfacto-habenular tract (Herrick, 1921, 1933, 1948) or the 'radix olfactorius medialis' of Snessarew (1908) is practically identical with the anterior olfacto-habenular tract described in reptiles. There is no suggestion in the published accounts, however, that it might be a crossed secondary olfactory tract. An interbulbar connexion, crossing in the habenular commissure, has been described e.g. in the frog by Snessarew (1908), in anurans by Röthig (1926) and in *Salamandra* by Kreht (1930). These fibres, however, pass caudally in a dorso-lateral olfactory tract, which appears to be equivalent, in part, to the dorsal division of Herrick's anterior olfacto-habenular tract and quite different in its course from the anterior olfacto-habenular tract of reptiles. According to Herrick, they arise very largely in the anterior olfactory nucleus. Apart, therefore, from the fact that certain authors have described secondary fibres as crossing in the habenular commissure of various amphibians, and the striking similarity between the anterior olfacto-habenular tract of *L. viridis* and the ventral division of anterior olfacto-habenular tract of amphibians (Herrick), no similarity to the lacertilian condition has been found. All these descriptions of the amphibian forebrain have been based on normal material and it seems possible that an experimental investigation might show a condition similar to or identical with that described in *L. viridis*; but at present this cannot be assumed.

So far as mammals are concerned, no reference has been found to any direct connexion from the olfactory bulb to the diagonal band or stria medullaris. The only suggestion of any resemblance to the condition in *L. viridis* as regards a crossed system of secondary olfactory fibres is found in Loo's (1931) account of the forebrain of the opossum. He described a component of the stria terminalis (stria terminalis 5) which joins the stria medullaris, and observed that its course and relations closely resembled those of the ventral division of Herrick's (1927) anterior olfacto-habenular tract, which is certainly comparable in its course to the anterior olfacto-habenular tract of *L. viridis*. Johnston (1923) identified this tract (Loo's stria terminalis 5) in the opossum and in the rabbit, but it must also be said that none of the more recent experimental studies in mammals has provided any evidence that these fibres are of bulbar origin.

In view of the very incomplete nature of the evidence, it is impossible to draw any general conclusions from this survey with confidence. It may be, since a component of secondary olfactory fibres has been found in the stria medullaris and habenular commissure in several amphibians but not in mammals, that modern reptiles present an elaboration of a fundamentally amphibian condition. The mammals may have arisen independently from generalized amphibian ancestors, and evolved along quite different lines, as suggested by Armstrong (1950) on the basis of his study of the optic system. So far as the olfactory system is concerned, this can be no more than a reasonable speculation, which must await further investigations before much weight can be given to it.

Finally, this work again emphasizes the importance of the experimental analysis of fibre systems, not only in mammalian but also in more primitive vertebrate brains. In addition, it demonstrates some of the limitations and difficulties of the particular method employed. It appears that different fibre systems react very differently to injury so far as concerns the rate of degeneration and the duration of the period during which degenerative changes in axons are demonstrable. It is probable that individual fibres in an apparently homogenous system do not all react at exactly the same time; and there may well be important differences between species in this respect. It follows that it is impossible to generalize from a set of observations which is limited to a single system and a single species; e.g., previous work (Goldby, 1937; Armstrong, 1950) suggested that both myelin sheath and axonal changes would occur more slowly in reptiles than in mammals. This does not appear to be generally so, since many secondary olfactory fibres disappear extremely quickly and, indeed, more quickly than those in the rabbit (Clark & Meyer, 1947).

The short duration of demonstrable changes and the variations in the time after the injury at which they appear make 'false negative' observations a very real possibility. The demonstration of degenerative changes or of fibre loss, if carefully controlled by comparison with normal material, should form a reliable basis on which to form conclusions. An apparent absence of any change may indicate only that too few fibres have disappeared, or show demonstrable changes, to be noticeable; or that the particular specimen has been prepared before the time when changes of a demonstrable kind occur. For this reason the reported observation that no degenerative changes or fibre loss were seen in the hippocampal cortex, septum and habenular nuclei cannot be taken as establishing more than a *prima* *facie* case that they receive no secondary olfactory fibres. If these structures do receive such fibres, it is probable that they are few in number, but even so it does not necessarily follow that they are, on that account, functionally insignificant.

One may conclude that, in using techniques which rely on the demonstration of axonal degeneration, it is necessary to investigate carefully the nature and time relationship of these changes, not only for a particular species but also for the particular system of fibres which is under investigation; and that this must be done before the observation of the presence or absence of these changes can be used as a reliable basis for anatomical interpretation.

SUMMARY

The connexions of the olfactory bulbs in *Lacerta viridis* have been investigated by studying the degenerative changes which occur after removal of the olfactory bulbs and other lesions. Nonidez's silver impregnation technique was used.

Degenerative changes were found to be of the same general type as in other similar investigations, but to occur more quickly than usual, viz. during the first 3 days after operation. In specimens killed after longer intervals the affected fibres had disappeared completely.

Observations, primarily of a loss of fibres following bulbar ablation, led to the following conclusions:

(*a*) The lateral olfactory tract receives fibres from both main and accessory olfactory bulbs; those from the main bulb end in the anterior olfactory nucleus and the most anterior part of the pyriform cortex; those from the accessory bulb pass to the anterior half to two-thirds of the pyriform cortex and to the amygdaloid nuclei.

(*b*) The intermediate olfactory tract arises from the main olfactory bulb and its fibres end over the surface of the olfactory tubercle.

(*c*) The medial olfactory tract can be divided into dorsal and ventral parts.

The dorsal part arises mainly in the accessory olfactory bulb and ends in the anterior olfactory nucleus. No evidence was found that any fibres of bulbar origin end in the septum or hippocampal cortex.

The ventral part runs caudally to reach the region of the diagonal band behind the olfactory tubercle. It passes laterally in the diagonal band and can be traced through the stria medullaris lateralis to the habenular commissure. After crossing in the commissure the fibres continue forwards in the stria medullaris of the opposite hemisphere to become the tract which in previous descriptions has been called the anterior olfacto-habenular tract. This tract is therefore a crossed secondary olfactory tract which runs rostrally to end in or near the anterior olfactory nucleus. This crossed tract originates in the main olfactory bulb.

(*d*) The diagonal band in *L. viridis* consists largely of the secondary olfactory fibres just described; it contains few if any fibres which connect medial and lateral centres in the hemisphere.

It is concluded that a crossed secondary olfactory tract, similar to that demonstrated in *L. viridis*, may be present in reptiles generally and possibly in Amphibia, but that further experimental work is necessary before this conclusion can be made definite.

This work has been carried out under the direction of Prof. F. Goldby of the Department of Anatomy, St Mary's Hospital Medical School. I wish to express my thanks to him and to Mr J. A. Armstrong for advice and criticism which they have given throughout.

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EXPLANATION OF PLATES

All figures are photographs of sections of the brain of *Lacerta viridis* prepared by Nonidez's silver impregnation method.

Plate 1

(Magnification in figs. 1 to 4, × 80.)

Fig. 1. Transverse section through the septal region of a normal brain to show the anterior olfacto-habenular tract present bilaterally.

Fig. 2. Transverse section through the same region 7 days after unilateral bulbar ablation. The anterior olfacto-habenular tract is present only on the side from which the olfactory bulb was removed.

Fig. 3. Transverse section through the diagonal band region to show the anterior olfacto-habenular tract present bilaterally in the normal brain.

Fig. 4. Transverse section through the same region 7 days after unilateral bulbar ablation (the same specimen as in fig. 2). The anterior olfacto-habenular tract is present only on the operated side.

PLATE 2

Fig. 5. Horizontal section passing through the stria medullaris lateralis of the unoperated side 3 days after unilateral bulbar ablation. (× 150.) A small area of fibre loss is seen in contact with the anterior margin of the optic tract. Compare with figs. 6 and 7.

Fig. 6. Horizontal section passing through the same region of the operated side in the same specimen as in fig. 5. (× 150.) A crescentic area of fibre loss is seen separated from the anterior margin of the optic tract by a small bundle of fibres. Compare with figs. 5 and 7.

Fig. 7. Horizontal section passing through the region illustrated in figs. 5 and 6, but in a normal brain. (× 150.) Compare with figs. 5 and 6.

Fig. 8. Horizontal section cutting the diagonal band region tangentially in a normal brain. (× 100.)

Fig. 9. Horizontal section, similar to fig. 8, on the unoperated side 3 days after unilateral bulbar ablation (the same specimen as in figs. 5 and 6). (× 100.) The section shows a loss of fibres from the medial and posterior part of the diagonal band system (the anterior olfacto-habenular tract); the remaining fibres are chiefly those of the medial olfactory tract (ventral part).

Fig. 10. The corresponding section to the one illustrated in fig. 9, but from the operated side. (× 100.) The presence of an intact anterior olfacto-habenular tract contrasts strongly with its absence on the opposite side (fig. 9). Loss of fibres from the medial olfactory tract, ventral part, though definite, is less pronounced than in most specimens.

PLATE 3

Fig. 11. Axonal varicosities in the anterior olfacto-habenular tract 3 days after division of the habenular commissure. (× 1400.)

Fig. 12. Small axonal varicosities (solid and vesicular) in the superficial part of olfactory tubercle 48 hr. after removal of main and accessory olfactory bulbs (L.V. 52). Similar structures were seen throughout the superficial layer of the pyriform cortex and in the anterior olfactory nucleus in this specimen. (× 1200.)

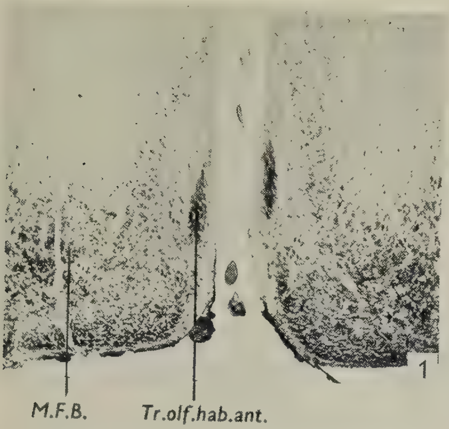
Fig. 13. Axonal varicosity and two (possibly terminal) vesicular swellings in the superficial layer of the olfactory tubercle in the same specimen as in fig. 12. (× 1200.)

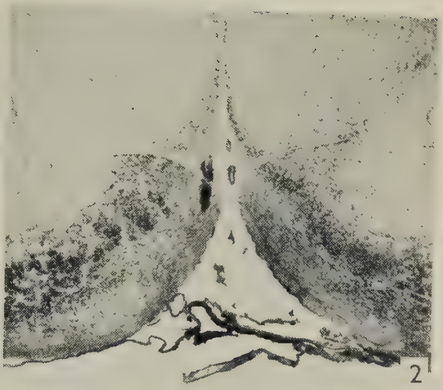
Fig. 14. Large solid axonal swellings on fibres of the ventral part of the medial olfactory tract 54 hr. after bulbar ablation (L.V. 69). These fibres are typical of those found also in the intermediate and lateral olfactory tracts in this specimen. (× 1200.)

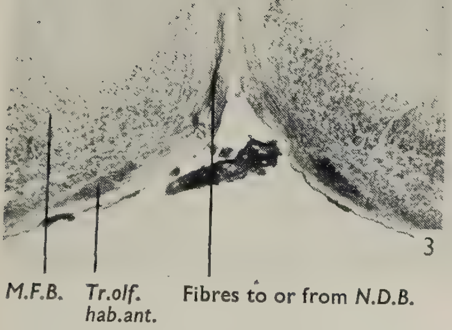
Fig. 15. Low-power photograph of the region from which figs. 12 and 13 were taken, to show the extensive nature of the degenerative changes. (× 450.)

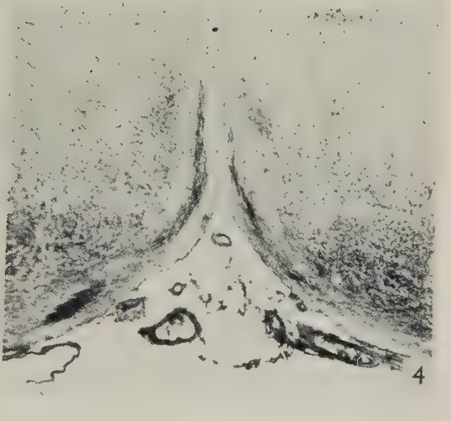
List of Abbreviations

D.B.	Diagonal band
L.F.B.	Lateral forebrain bundle
M.F.B.	Medial forebrain bundle
N.D.B.	Nucleus of diagonal band
N.supraop.	Nucleus supraopticus
St.med.lat.	Stria medullaris lateralis
Tr.olf.med., pars vent.	Tractus olfactorius medialis, pars ventralis
Tr.olf.hab.ant.	Tractus olfacto-habenularis anterior
Tr.opt.	Tractus opticus

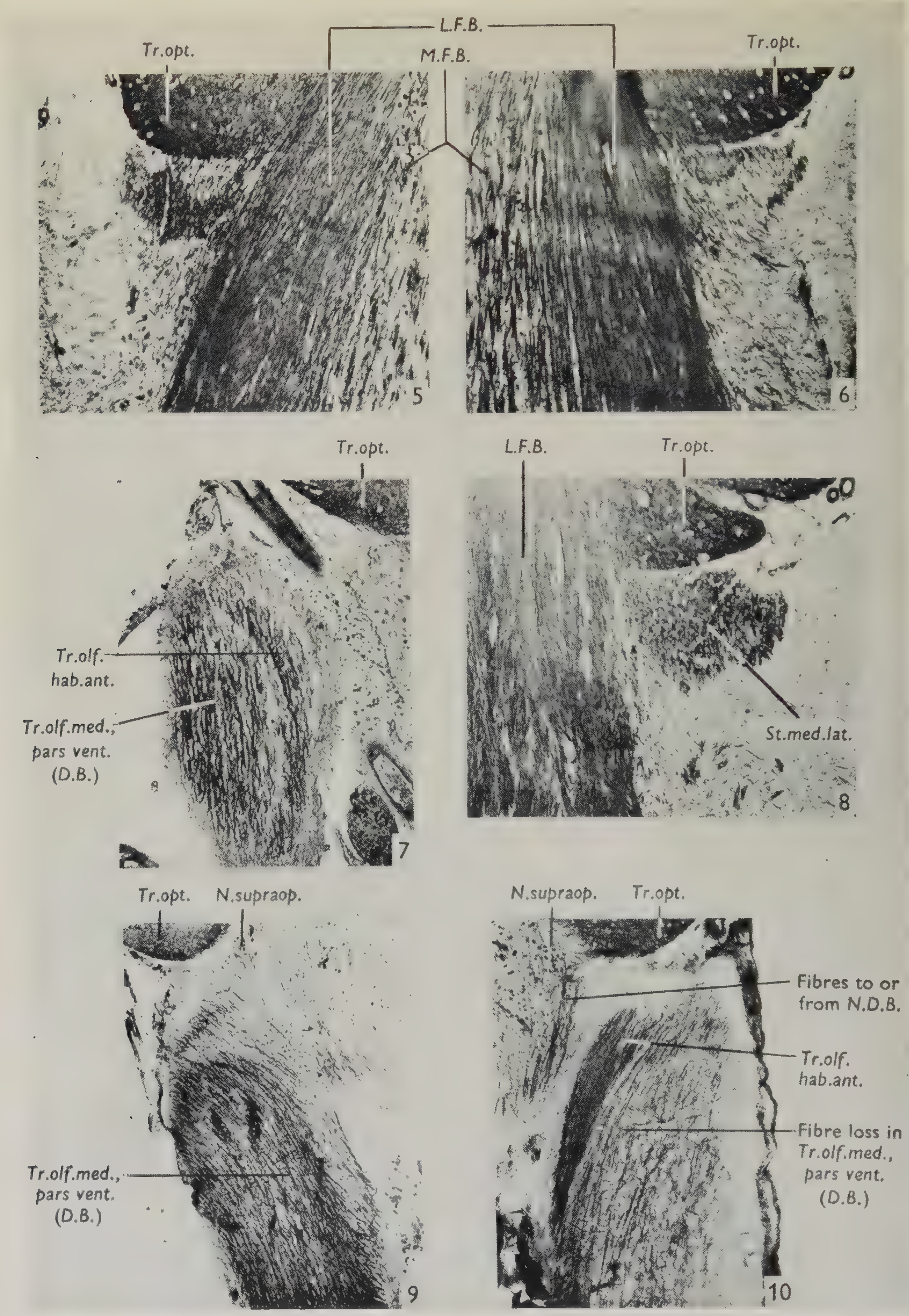




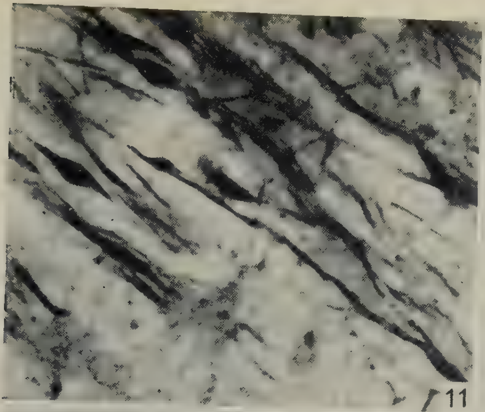




GAMBLE—Secondary olfactory connexions in *Lacerta viridis*



GAMBLE—SECONDARY OLFACTORY CONNEXIONS IN *LACERTA VIRIDIS*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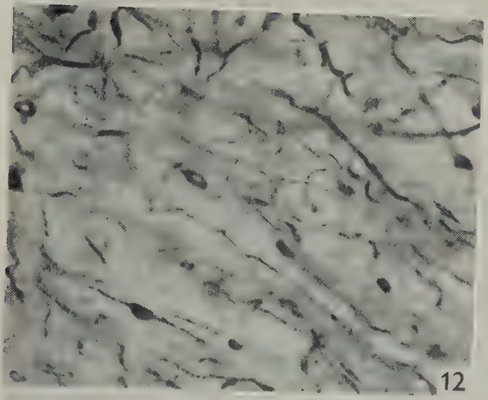
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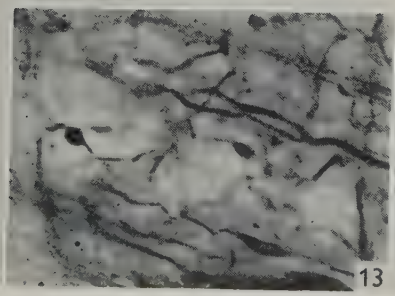
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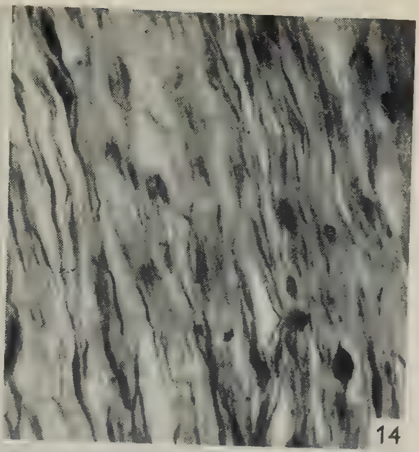
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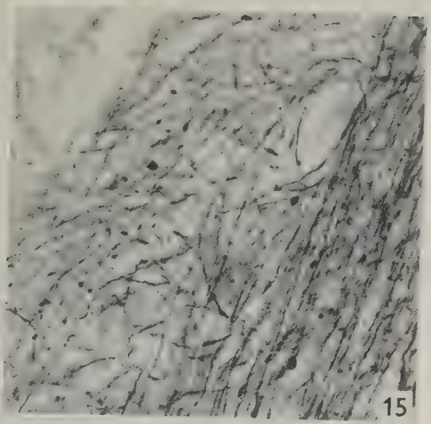
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GAMBLE—Secondary olfactory connexions in *Lacerta viridis*

THE EFFECT OF THE DEGREE OF ELBOW FLEXION ON THE MAXIMUM TORQUES DEVELOPED IN PRONATION AND SUPINATION OF THE RIGHT HAND

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This work is a continuation of a previous investigation made by Darcus (1951) on the effect of different hand, elbow and shoulder positions on the maximum isometric torques that can be exerted in attempted pronation and supination of the right hand.

METHODS

The maximum torques that can be developed isometrically in attempted pronation and supination were determined with the hand in six positions between full pronation and full supination. The measurements were carried out with the arm adducted and with the elbow flexed to 150° (A), 90° (B) and 30° (C) (full extension of the elbow $\equiv$ 180°).

The position of the hand with the palm facing medially was taken as 0°. Angular displacements of pronation were recorded as minus and of supination as plus. Data were obtained with the hand at -60, -30, 0, $+30$, $+60$ and $+90°$. All these positions fell within the full range of movement of each subject.

The subjects were three naval ratings, ages 18, 19 and 27 years, with no apparent physical defect. Each was right-handed. They had had no previous experience of the apparatus.

All hand positions in each of the three elbow positions were studied in all three subjects on each of 6 consecutive days. The order in which the hand positions and the direction of the attempted movement were taken was randomized. Half an hour was allowed between the runs in each different elbow position.

The torques were recorded by means of an electrical strain-gauge dynamometer (Darcus, 1951).

RESULTS

The effect of hand position on the maximum torques developed in attempted pronation and supination. The results of the present series of experiments confirm that there is a linear relationship between the position of the hand and the isometric torque developed, and that as the position of the hand alters in the direction of supination, so the isometric pronation torque increases and the supination torque decreases (Fig. 1). In one individual there is an exception to this general finding; with the elbow flexed to 30°, there was found to be no significant correlation between the torque of supination and the position of the hand. Although this linear relationship generally exists, it can be seen from graphs drawn from the raw data (Fig. 1) that the slope of the curves is steeper towards the full pronation end of the pronation curves and towards the full supination end of the supination curves. In both the pronation and supination curves, there is a sharp change of slope at hand position +30°. This, however, is more marked in pronation.

For each subject, the slopes of the pronation curves are steeper than those of the corresponding supination curves. The slopes of the curves, both for pronation and supination, are greatest when the elbow is flexed to 150°, and least when the elbow is flexed to 30° (Fig. 1 and Table 1).

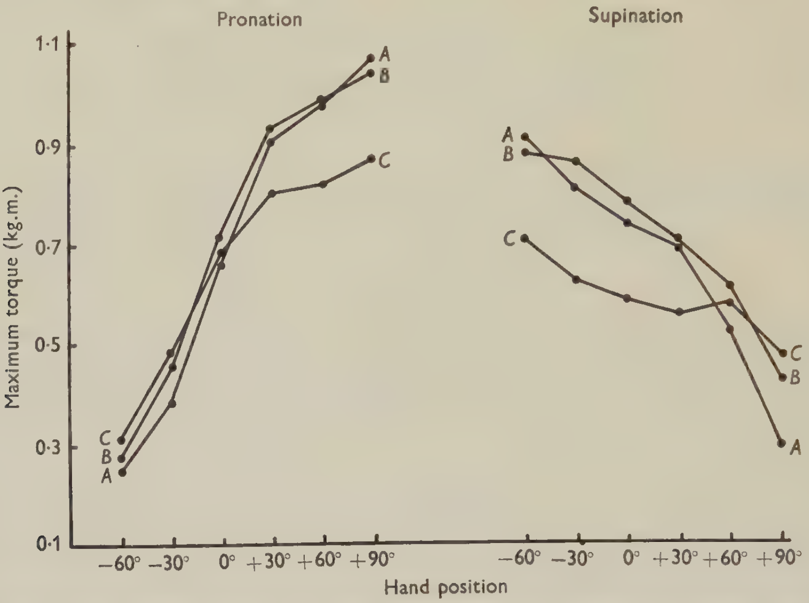

Fig. 1. Maximum torques exerted in different hand positions. Average of eighteen observations on the three subjects: (A) elbow flexed to 150°, (B) to 90°, and (C) to 30°.

Table 1. *Average difference between torques exerted in successive hand positions*

Subject	Flexion of elbow (°)	Pronation (kg.m.)	Supination (kg.m.)
I	150 (A)	0·147	0·123
II	150	0·167	0·108
III	150	0·207	0·109
I	90 (B)	0·132	0·102
II	90	0·159	0·091
III	90	0·190	0·073
I	30 (C)	0·054	0·001
II	30	0·105	0·048
III	30	0·174	0·060

The effect of elbow position on the average maximum torques in attempted pronation and supination. As discussed in the previous paper, a more representative estimate of the relative strengths of pronation and supination in the different elbow positions may be obtained by calculating from the regression lines the torques at the mid-point of the full range of movement (Table 2). As the mid-point of the range tested (i.e. +15°) does not differ greatly from the mid-point of the full range in any elbow position, the results obtained from the raw data are essentially the same (Table 2).

It can be seen in Fig. 2 that the strongest position for pronation and for supination is with the elbow flexed to 90° and the weakest with the elbow flexed to 30°, with one exception. This is in subject II, in which the pronation torque is smallest with the elbow flexed to 150°, but the difference between the torque in this position and that with the elbow flexed to 30° is small (0·01 kg.m.) and not significant.

The degree of elbow flexion has a much more pronounced effect on the supination torque than on the pronation torque. The average difference between the average supination torques in each of the elbow positions is double that between the average pronation torques.

Table 2. *Average torque of pronation and supination for the range of hand positions tested and at the mid-point of the full range of movement*

		Range tested (−60° to +90°)			Mid-point of full range			
		Average torque			Average torque			
ect	Elbow flexion (°)	Pronation (kg.m.)	Supination (kg.m.)	Percentage difference (S≡100)	Pronation (kg.m.)	Supination (kg.m.)	Percentage difference (S≡100)	Mid-point of full range (°)
	150 (A)	0·69	0·60	15·0	0·69	0·59	16·9	+14·5
	90 (B)	0·67	0·69	−2·9	0·69	0·68	1·5	+18·0
	30 (C)	0·61	0·58	5·2	0·62	0·58	6·9	+21·0
	150 (A)	0·56	0·54	3·7	0·55	0·55	0	+12·0
	90 (B)	0·61	0·60	1·7	0·62	0·60	3·3	+16·5
	30 (C)	0·54	0·49	10·2	0·56	0·48	16·7	+23·0
	150 (A)	0·87	0·83	4·8	0·93	0·81	14·8	+20·0
	90 (B)	0·92	0·83	10·8	0·94	0·82	14·6	+19·5
	30 (C)	0·84	0·69	21·7	0·89	0·68	30·9	+23·0
ns	150 (A)	0·71	0·66	7·4	0·72	0·65	11·2	—
	90 (B)	0·73	0·71	3·7	0·75	0·70	7·1	—
	30 (C)	0·66	0·59	13·0	0·69	0·58	19·0	—

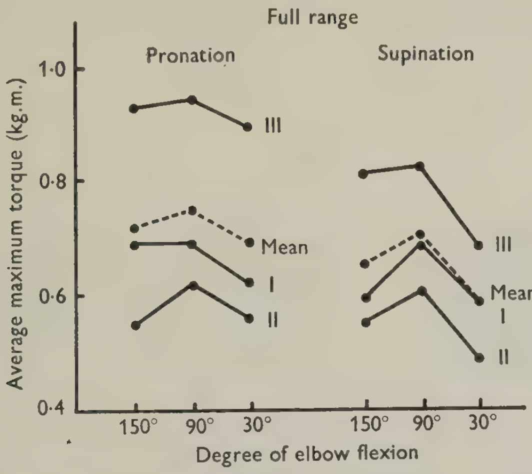

Fig. 2. The estimated average maximum torques at the mid-point of the full range of movement in the three elbow positions for each subject.

Differences between the force of pronation and supination. At the mid-points of the full range in the three elbow positions in the three subjects, pronation is stronger than supination in eight cases and the same in one. In general, the greatest differences are found when the elbow is flexed to 30° and the least when it is flexed to 90°. The estimated positions of the hand at which the torque of pronation equals that of supination are given in Table 3.

Variation in the maximum torque developed. If the average maximum pronation or supination torque recorded in each run is expressed as the percentage of the average of the six runs for each elbow position in each subject, it can be seen that there are marked variations in these percentage torques on different occasions (Fig. 3). This figure indicates that, considering all elbow positions, there is neither a consistent increase nor decrease of statistical significance in any of the subjects during the experimental period. The same applies to the torque exerted in each elbow position in the same individual on successive days. Neither is there any correlation between the pronation or supination torques exerted and the order of the runs. Furthermore, in each elbow position, no relationship exists between the percentage pronation and supination torques. For example, if in any one run, that for pronation is greater than the average for the six runs, then that for supination does not necessarily vary in the same direction.

Table 3. *The hand position at which the torque of pronation equals that of supination (estimated from the regression lines)*

Degree of elbow flexion	Subjects I	II	III
150	+5	+12	+10
90	+16	+12	+4
30	+1	+3	+6

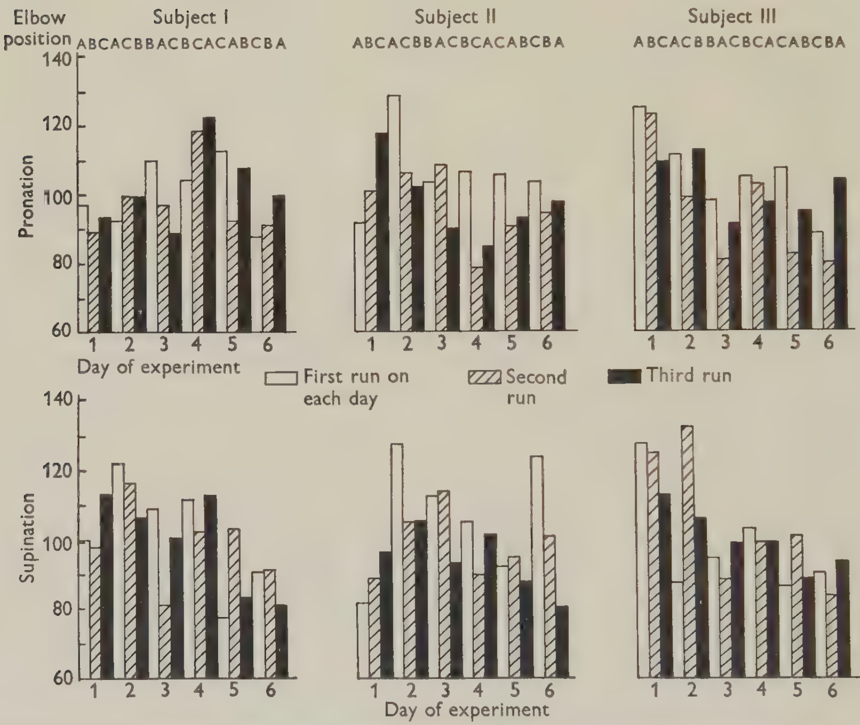

Fig. 3. The total maximum torque for each run in each elbow position expressed as a percentage of the average for each position.

In each of the elbow positions, there was marked variation in the readings obtained in each of the hand positions on successive occasions. These variations were greatest in the weakest hand positions.

DISCUSSION

Although a somewhat larger range of hand positions was tested, the results of these experiments concerning the linear relationship between isometric torques and hand positions, the relative strength of pronation and supination and the variations found in the maximum torques exerted, confirm those found in the previous investigation (Darcus, 1951). The results obtained in each of the experiments with the shoulder adducted and the elbow flexed to 90° are similar (Table 4).

Table 4. *A comparison between the results with the shoulder adducted and the elbow flexed obtained in the present investigation* (*A*) *and those obtained by Darcus* (1951) (*B*).

Average torque at mid-point of full range		Percentage difference (S≡100)	Average difference of torque in successive hand positions		Mid-point of full range of movement (°)	Hand position in which pronation = supination torque (°)
Pronation (kg.m.)	Supination (kg.m.)		Pronation (kg.m.)	Supination (kg.m.)		
0·75	0·70	7·1	0·160	0·089	21	12
0·78	0·69	15·0	0·219	0·132	18	11

The effect of the degree of elbow flexion on the maximum torque. The results show that, of the positions of elbow flexion studied, that in which the elbow is flexed to a right angle is the strongest, both for pronation and for supination. This may be accounted for by the fact that the mechanical advantage of the humeral head of pronator teres, of biceps and of the humeral head of supinator is maximal near this degree of flexion; the mechanical advantage decreasing on either side of this point (Fick, 1911). The fact that the strength of pronation and supination is greatest in the position of rectangular flexion confirms the statements that appear in text-books of anatomy (Morris, 1902; Bryce, 1923; Walmsley, 1934; Steindler, 1935).

Of the other two positions studied, the finding that pronation and supination are stronger at 150° flexion than at 30° flexion may be explained by the fact that, although the mechanical advantage of the muscles is similar, when the elbow is flexed to 30° their length is shorter and their contractile force is therefore smaller.

The fact that elbow flexion has a greater effect on the strength of supination than on that of pronation may be explained by referring to the muscles producing these movements. Pronation is produced mainly by the humeral and ulnar heads of pronator teres and by pronator quadratus. Of these, only the humeral head of pronator teres is affected by elbow flexion. Supination is produced mainly by the humeral and ulnar heads of supinator and by biceps. Of these, biceps and the humeral head of supinator are affected by elbow flexion. Thus more of the muscles concerned in supination are affected by varying degrees of elbow flexion than are those in pronation.

No explanation is offered for the greater differences between the pronation torques exerted in successive hand positions than between the supination torques, nor for the fact that the differences of torque between successive hand positions for both pronation and supination increase as the elbow is flexed.

It has been calculated that, with the elbow in various degrees of flexion, the pronation and supination torques are equal when the hand position (as measured from the plane of the hand-grip) is on the average 8° towards the supination side of the neutral point. Although no precise data are available, it can be shown that, when the hand is in this position, the distal ends of the radius and ulnar are in approximately the same vertical plane. Thus, it may be demonstrated in the light of further work that, in general, the power of pronation and supination with the elbow flexed is equal when the distal ends of the radius and ulna are in the same vertical plane.

SUMMARY

1. The torque exerted in attempted pronation and supination of the right hand was studied in three subjects, with the shoulder adducted, in different positions of the elbow and hand.

2. The linear relationship between hand position and the maximum pronation and supination torque was confirmed, although the raw data indicate that the slope of the curves is steeper towards the full pronation end of the pronation curves and towards the full supination end of the supination curves.

3. In all subjects, both pronation and supination are strongest with the elbow flexed to 90° and generally weakest with the elbow flexed to 150°. The differences in the torques exerted in different elbow positions were more pronounced in supination than in pronation.

4. At the mid-point of the full range of movement, pronation was stronger than supination in eight cases and the same in one. They are equal when the hand is on the average 8° towards the supination side of the mid-point. This may correspond to the position in which the distal end of the radius and ulna are in the same vertical plane.

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THE REPRODUCTIVE TRACT AND THE PLACENTA AND MEMBRANES OF PÈRE DAVID'S DEER (*ELAPHURUS DAVIDIANUS* MILNE EDWARDS)

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INTRODUCTION

The placentae and gravid uteri of some Cervidae have been described (Turner, 1878, 1879; Strahl, 1906; Kolster, 1909; Andresen, 1922) and the previous literature has been reviewed by Andresen (1927). A preliminary account has been given by us (1951) of placentation in Père David's deer (*Elaphurus davidianus*). We have been unable to trace any detailed account of the placentation of this animal.

As far as can be ascertained, Père David's deer is now extinct in the wild state and is only found as a large herd in Woburn Park, and as small groups or individuals in certain Zoological Gardens. The Duke of Bedford (1947) has given an account of the discovery of this deer in 1865 by the missionary, Père Armand David, in the Imperial Hunting Park of Pekin. A more detailed account of the discovery is given in a recent paper by Dobson (Wood Jones, 1951). According to Whitehead (1950), its native name appears to have been *Mi-lou*, *Sen-pou-siang* or *Ssu pu-hsiang*. From 1869 onwards some specimens reached Zoological Gardens in Europe where they occasionally bred. In 1894 the herd of the Imperial Hunting Park escaped and were killed when the walls of the Park were breached by the flooding of the Hun-Lo river. In 1898 the first pair of deer arrived in Woburn Park and in due course began to breed. Other animals were imported from other stocks then existing on the Continent. The deer on the Continent have since died. It is undoubtedly due to the interest of both the late Duke and the present Duke of Bedford that the species is not totally extinct to-day.

Mating takes place in June and July (i.e. 2–3 months earlier than the red deer), and parturition has been reported in early April, but the usual date is mid-April to May; the latest date recorded for parturition was in September with a twin pregnancy.

There has been much discussion as to the systematic position of the Père David deer. Milne Edwards (1866*a*, *b*), Gray (1872) and Fitzinger (1874) regarded it as a distinct genus. Gray believed that its position was 'between the roe and the American deer', whereas Fitzinger thought it was related to the rusine deer. Sclater (1871) and Brooke (1878) included it in the genus *Cervus*. Cameron (1892), basing his conclusions on the structure of the antlers, believes that it is a separate genus unrelated to any of the Old-World deer, with the possible exception of the roe deer (*Capreolus capreolus*), and closely related to the American deer (Odocoileinae). For a discussion of other features, see Lydekker (1898) and Simpson (1945). The Duke of Bedford is of the opinion that it is a distinct genus of the Cervidae.

MATERIALS AND METHODS

The material at our disposal consists of four specimens (PD 1–4) of the gravid uterus, the ovaries, and parts of the vagina, and two specimens (PD 6 and 5) of the genital tract from non-pregnant animals. There was no evidence from their behaviour that either of the non-pregnant animals was in oestrus. In the first of these non-pregnant specimens neither ovary contains a corpus luteum but each contains a large number of healthy and atretic follicles. The right ovary of the other non-pregnant animal contains a corpus luteum. The genital tracts of the two female foetuses were also examined.

The first specimen (PD 1) was from a hind, 10 years old, killed on 13 February 1950. The uterus contained a female foetus, C.R. length 38 cm., which weighed 3·1 kg. after fixation. The entire specimen was fixed in 10 % formalin. The second hind (PD 2), age unknown, was killed on 10 January 1951 and contained a female foetus, C.R. length 36 cm., which weighed 2·52 kg. after fixation. Before fixation, the umbilical arteries and veins were injected with coloured gelatin. One placentome* had already been removed and portions were fixed in Zenker-formalin and Bouin's fixative. The third hind (PD 3) was killed on the same day as the second. It contained a male foetus, C.R. length 33 cm., and weighing after fixation 1·98 kg. The maternal uterine arteries and veins were injected with coloured gelatin, and the whole specimen was subsequently fixed in 10 % formalin. The fourth hind (PD 4) was killed on 9 March 1951; it contained a male foetus, C.R. length 27·5 cm., which weighed 1·07 kg. after fixation. The uterus was opened immediately after removal from the hind, and portions of the uterine wall, chorion and placentome were at once placed in cold 80 % alcohol, acetone, Rossman's fluid, and 4 % basic lead acetate respectively. Frozen and paraffin sections were subsequently prepared from both the injected and the uninjected material. It was found advantageous to cut sections of the whole placentome which were mounted on large glass slides and covered with sheets of mica. Such treatment facilitated examination of the relations of foetal and maternal tissues. The two non-pregnant females were killed on 15 August 1951. One of them had ovulated (PD 5) and so the uterine tubes and uterus were flushed with normal saline, but no products of conception were recovered. The second female (PD 6) had not ovulated.

A variety of staining techniques and ancillary methods were used. Sections were stained with Harris's or Heidenhain's haematoxylin and eosin, phloxin or Van Gieson's stain, Mallory's triple stain, Heidenhain's azan stain, Masson's stain, eosin-methylene blue, toluidin blue, and pyronin-methyl green stains. Robb-Smith's modification of Foot's method and Rennie's method for reticulum were used. Sections were stained for glycogen and other polysaccharides with Best's carmine, by the Bauer-Feulgen and the McManus periodic acid method. The Feulgen method for the demonstration of desoxyribonucleic acid was also carried out. Alkaline and acid phosphatases were demonstrated by the modifications of Gomori's method used by Wislocki & Dempsey (1946). Sections were also stained with the Turnbull blue method for the presence of iron. Frozen sections were stained with Sudan III and Sudan Black and also with the Schiff reagent. Use was also made of the fluorescent, polarizing and phase-contrast microscopes.

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* The terms cotyledon and placentome have caused some confusion, sometimes having been incorrectly used synonymously. The placentome is composed of two elements, the maternal caruncular tissue, and the foetal chorionic tissue. We have restricted the use of the term cotyledon to the patches of chorionic villi.

In addition, observations were made on a number of whole mount preparations. Foetal villi were carefully dissected from the maternal crypts and examined unstained or stained under the binocular dissecting microscope or the phase-contrast microscope. Whole mounts also were made of the injected material and corrosion casts of the specimens injected with latex were prepared. Comparison of the appearances in the stained sections with those seen in the injected specimens enabled a more accurate interpretation of the vascular supply of a villus to be made. The injection masses consisted of 15 % gelatin to which had been added Monastral fast blue B.N.S. or Monastral fast scarlet R.N.V.S., or latex containing one of the above dyes. These dyes were kindly given by I.C.I. Ltd.

Use was also made of spreads of chorion, with the allantois detached, in order to study the areolae in the extra-cotyledonary areas. Such spreads can be readily examined unstained or stained (see Wimsatt, 1950).

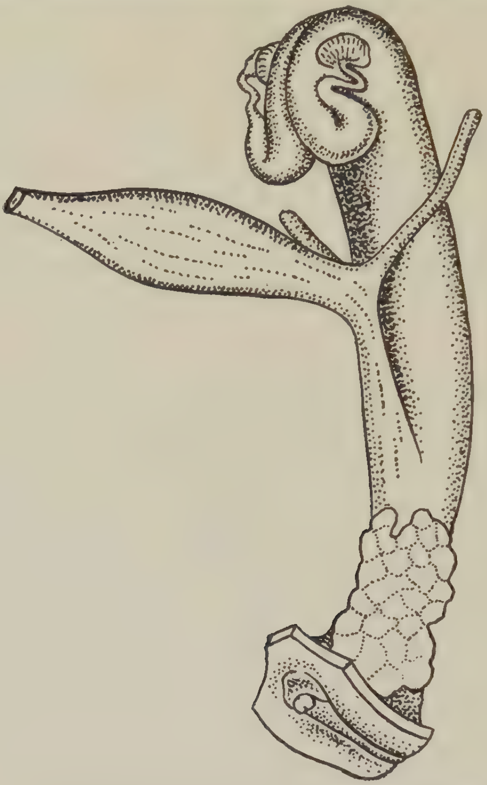
THE GENITAL TRACT OF THE NON-PREGNANT ANIMAL

The general macroscopic appearances of the genital tract of the two non-pregnant females are essentially similar except for a slight variation in the form of the caruncles. The uterus is typically bicornuate, but is markedly anteflexed at the level of the fused horns, so that the morphological dorsal surface of each horn faces towards the anterior abdominal wall (Text-fig. 1; Pl. 2, fig. 5). This anteflexion of the uterus has been found by us in a number of other species of deer. Each horn turns sharply laterally soon after it has separated from its fellow; it then narrows rapidly, and turning cranially and medially becomes continuous with the convoluted uterine tube. The ovaries are attached closely to the side walls of the uterus approximately at a level opposite the caudal end of the fused horns. During pregnancy dilatation of the uterus occurs ventralwards and the ovaries of the pregnant uterus are found attached to the dorso-lateral surface.

In the adult, each horn measures approximately 15 cm. in length and is 2·5 cm. in diameter at its widest point; the body of the uterus measures 4 cm. in diameter. The cervix is 4–5 cm. in length and 2–4 cm. in diameter, and projects into the vagina in the form of a cone. Within the cervical canal there are three to four transverse folds which project towards the external os. Both the external os and the cervical folds are grooved by numerous deep longitudinal clefts (Text-fig. 2).

The vagina is 15–20 cm. in length, 5–6 cm. across and 4–5 cm. in depth. Posteriorly, at the opening of the vagina, the walls are thrown into a number of longitudinal folds 3–4 cm. in length, 2–4 mm. across and projecting for 2–5 mm. into the lumen of the vagina. The opening of the urethra, circular in outline and 7 mm. in diameter, lies immediately ventral to the opening of the vagina. There are two folds, one on each side of the urethral opening, which unite ventral to the urethral opening to form a single fold which extends forwards and ventrally for 4–5 cm. to become continuous with the frenulum at the base of the clitoris. The clitoris is conical in shape, 11 mm. in length, has a marked hood-like prepuce, and from each side of it a narrow fold, 3–4 cm. in length, passes towards the urethral opening. The two folds diverge and diminish in size and finally disappear.

The uterus of the animal which had recently ovulated (PD 5) was opened by two incisions which passed along the morphologically dorsal aspect. The whole specimen was then laid flat and is illustrated in Text-fig. 2. The mucosa is thrown into a number of flattened quadrilateral folds in the fused and unfused portions of the horn. There are also a number of irregular transverse folds present both in the body and the horns of the uterus. There are three caruncles in the right horn arranged linearly along the morphological dorso-lateral aspect (Text-fig. 2). The most posterior caruncle is 25 mm. in length and 10 mm. thick, and projects for 10 mm. into the lumen of the uterus. The intermediate caruncle is 50 mm. in length and 9 mm. thick and projects into the uterine cavity for 11 mm. The third caruncle is 2·8 mm. in length, is 6 mm. across at its widest point and narrows to 3 mm. at its anterior extremity. It projects 6 mm. into the uterine lumen.

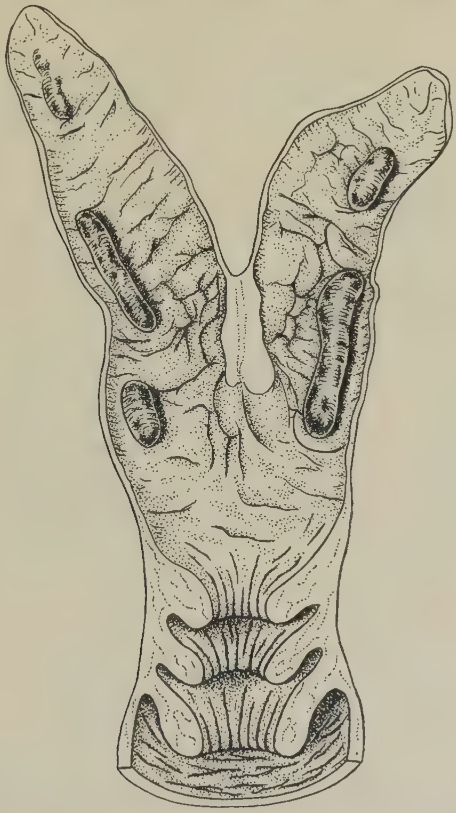


Text-fig. 1. A drawing of the genital tract of a 38 cm. foetus of *Elaphurus davidianus* (PD 1).

The left horn contains only two caruncles, the posterior of which is 50 mm. in length, 11 mm. across and projects for 10 mm. into the uterine lumen. The second caruncle is 25 mm. in length and 9 mm. across and projects for 10 mm. into the uterine lumen. All the caruncles (Text-fig. 2) are elongated, rounded at each end, and are attached by low pedicles some 5 mm. across. The posterior caruncles of the second specimen are more raised but narrower than in the first specimen (Pl. 2, fig. 6).

The uterine mucosa of the specimen PD 6 is 1–2 mm. in thickness. The mucosal and caruncular epithelium is columnar. The cells are 15μ high and fairly regularly arranged. The nuclei are situated in the middle third of the cells and are elongated and vesicular. Occasional darkly staining, small round cells, which are probably lymphocytes, are invading the base of the epithelium.

Immediately below the epithelium of the mucosa is a narrow zone only 0·1–0·2 mm. thick, composed of closely packed, darkly staining cells. The cells are particularly densely arranged nearest the epithelium and gradually become more loosely packed at the outer edge of this layer. There is an extensive capillary network within the layer which is most obvious about the ducts. Outside this narrow compact zone is a loosely arranged broader zone containing the glands and a large number of what appear to be obliterated and patent blood-vessels. The glands are somewhat tortuous and extend through this zone to the muscle layer. The glands average 60μ in diameter near the lumen of the uterus but are only 30μ in diameter near the muscle. The cells lining the glands are columnar and contain large darkly stained nuclei. Few of the glands have a marked lumen and there are no secretory products.



Text-fig. 2. A drawing of the inner aspect of the uterus of an adult *Elaphurus davidianus* (PD 6). The cervix and uterus have been opened out by incisions made through the morphologically dorsal aspect.

In the caruncle there is a smaller subepithelial compact zone containing large numbers of capillaries. Beneath this is a narrow zone of approximately the same thickness, consisting of more loosely arranged cells and also containing a number of pigment-laden histiocytes. Deep to this layer is the main body of the caruncle which in this animal almost entirely consists of the remnants of large sclerotic blood-vessels. There are no glands present in the base of the pedicle on which the caruncle is placed.

The uterine mucosa of the animal which had ovulated (PD 5) varies in thickness from 3 to 7 mm.; the columnar epithelium is 30 μ high and the nuclei are at the base. There is evidence of secretory products in the superficial part of the cell. The subepithelial zone is much denser than the outer zone, but its cells are swollen and the tissue is oedematous. There is considerable engorgement of the capillaries with red cells, and the whole subepithelial zone is hyperaemic. The remainder of the mucosa is also oedematous and in several places the stroma has a hyaline appearance. The glands are tortuous and coiled and extend to the muscle layer and in places the bases of the glands insinuate themselves between the muscular bundles. Near the lumen of the uterus the glands are 75 μ in diameter and their cells are columnar with all the nuclei at the base of the cell. The glandular cells show secretory activity and there are some secretory products in the lumen.

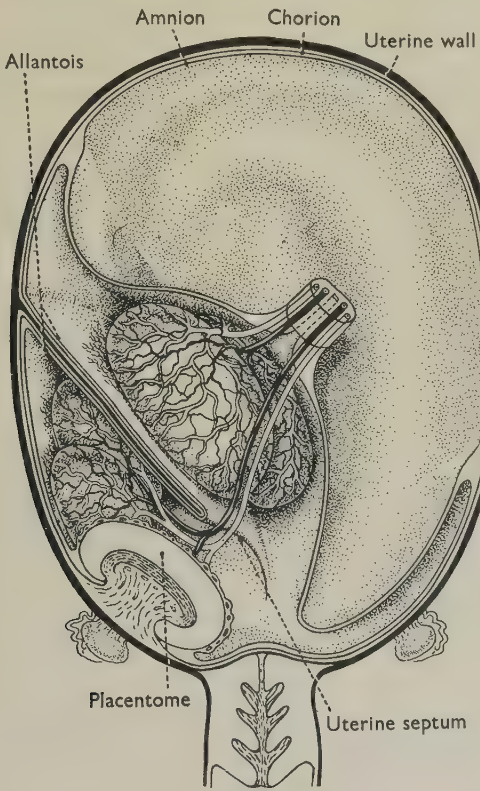
The epithelium covering the caruncle is pseudo-stratified and both the nuclei and cytoplasm stain more deeply that in the epithelial cells lining the mucosa; the nuclei are placed in the central third of the cell. The subepithelial zone is very vascular and contains a large number of engorged capillaries. The stroma is oedematous near the surface. The basal layer of the caruncle is denser and contains numerous obliterated arteries similar to, but smaller than, those described in the other non-pregnant animal (PD 5).

THE GRAVID UTERUS AND THE GENERAL ARRANGEMENT OF THE FOETAL MEMBRANES

In our youngest specimen (PD 4) of the gravid uterus, there was unequal expansion of the uterine horns. The right uterine horn was much enlarged, oval in shape and 45 cm. long by 35 cm. across; the left horn was a conical appendage some 15 cm. in length. The other three specimens were similar in appearance, each uterus in the form of a sphere 75–90 cm. in circumference; and, from the examination of the unopened uterus, it was impossible to recognize the separate horns (Text-fig. 3). The ovaries were found on the dorsal aspect some 7 cm. on each side of the cervix. Each tube passes posterior and then lateral to the ovary.

The vagina is 30–35 cm. in length and 5 cm. in diameter. Long ridges, 2–3 mm. high and equally broad, pass from the cervix down to the vaginal opening. Numbers of mucinous cysts are present at the opening. The cervix is 9 cm. in length and 6 cm. in diameter; it is extremely fibrous and the lumen is narrow. Three well-marked circular folds are found within the cervical canal. They project downwards, the most cranial being the smallest, and in section they are imbricated like tiles. Each fold has numerous longitudinal ridges on its inner aspect. These cervical folds are reminiscent of those found in a number of Cetacea (Harrison, 1949) and it is possible that their function may be to assist in retaining seminal fluid within the uterus.

On opening each uterus, the cavity was found to be unequally divided by a thin obliquely placed septum, 22–25 cm. in length, into a larger space containing the foetus and part of the chorionic sac and a smaller space containing the remainder of the sac (Text-fig. 3). The lower edge of the septum was crescentic in form. The tips of the crescent were placed some 3 cm. above the internal cervical os. Both of the cavities which represent the uterine horns and the common cavity of the corpus uteri below the septum were completely filled with the large chorionic sac. The sac bears five large placentomes (in the fourth specimen two small accessory lobules were present). In each specimen two large placentomes were found in the horn containing the foetus (right) and three smaller placentomes in the other (Pl. 1, fig. 1). Each placentome is convex, and ovoid in shape, typical measurements being 10–12 cm. in length, 6–8 cm. in thickness and 4–7 cm. in depth for the large placentome, and 8–6 cm. in length, 7–4 cm. in breadth and 4–3 cm. in depth for the smaller. In the fourth specimen the two small accessory placentomes measured 4 × 2 × 2 cm. and 2 × 2 × 2 cm.



Text-fig. 3. A drawing of the arrangement of the membranes and the cotyledons in the uterus of a pregnant *Elaphurus davidianus* (PD 1). The animal was pregnant in the right horn and the drawing has been made from the posterior aspect.

The placentomes of the right horn were attached to the posterior wall and to the septum, whereas those of the left horn lay mainly on the posterior wall.

The amnion occupied the greater part of the right horn and projected into the corpus uteri and for a short distance under the crescentic margin of the septum into the left horn. The amnion is adherent to the chorion on the convexity of the right horn. This undoubtedly corresponds to the upper part of the anti-mesometrial border of the uterus. It should be noted that this area is quite extensive owing to the great dilatation of the right horn. Elsewhere the amnion is separated from the chorion by the allantois.

In three of the specimens the inner aspect of the amnion exhibited a large number of discrete white plaques 2–3 mm. in diameter, the fourth exhibited equally numerous, firm, inwardly directed yellow papillae, some 3–5 mm. in length. Both the papillae and plaques were most numerous about the umbilical cord. Microscopical examination shows these plaques and papillae to be made of heaped-up squamous amniotic epithelium (Pl. 2, fig. 7). The amniotic fluid was light yellow in colour.

The general arrangement of the allantois is essentially similar in all four specimens. The main mass of the allantois is directed mesometrically, but it is reflected round the septum and over all the cotyledons (see Text-fig. 3). It was only loosely attached to the chorion, and could be stripped away quite easily both before and after fixation. In several places in the intercotyledonary area there were masses of white creamy material, sometimes weighing up to 10 g., situated between the chorion and the uterine mucosa. There is little doubt that this material is secreted by the uterine glands.

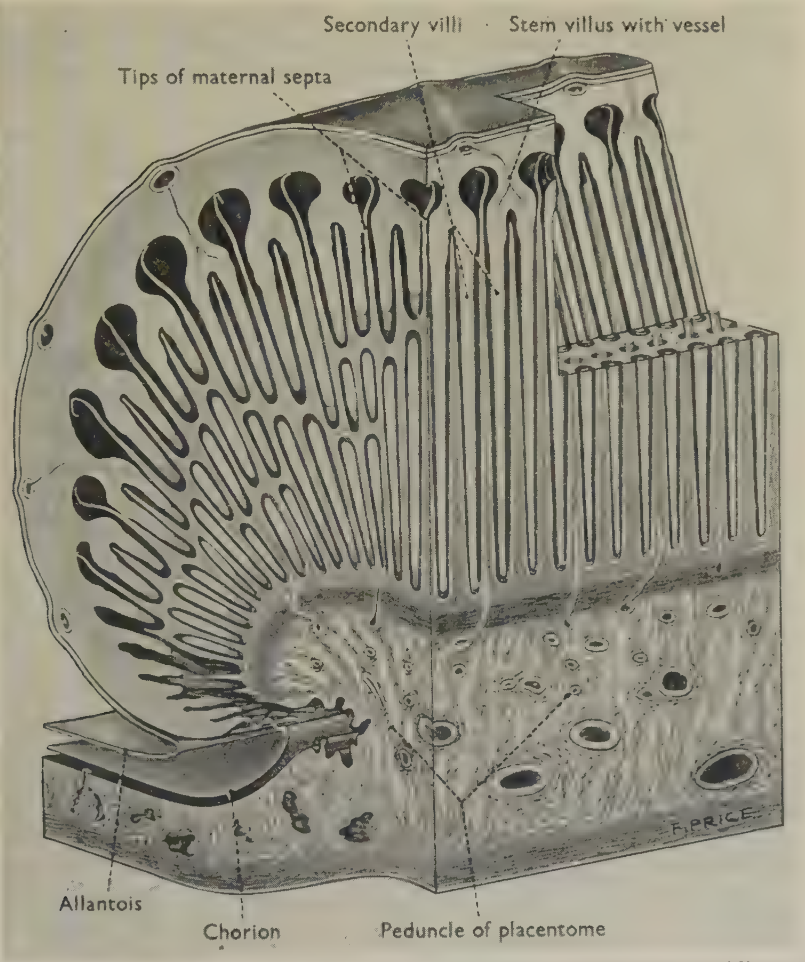
The umbilical cord

The cord is 10–12 cm. in length and 2½–3 cm. in diameter. It contains two umbilical arteries and two thick-walled veins. In addition there are numbers of allantoic vessels arising from the umbilical vessels in the substance of the cord. Further minute vessels arise from the umbilical vessels to supply the mesenchymatous tissue of the cord. Between the two arteries is the allantoic duct (Pl. 2, fig. 7; Text-fig. 3). One artery and vein pass direct to the two larger cotyledons in the right horn. The other artery and vein pass below the edge of the septum and supply the cotyledons of the left horn. There are extensive anastomoses through the chorionic vessels between the cotyledons of a horn and between those of the two horns.

THE MACROSCOPIC STRUCTURE OF THE PLACENTOMES

Each placentome is a large ellipsoidal mass lying close against the uterine wall (Pl. 1, figs. 1, 2). It is supported on a peduncle, or pedium, composed of caruncular tissue, which projects into the uterine aspect of the placentome. The surface towards the uterine lumen is convex. In a placentome measuring 9 cm. in length and 6 cm. across, the peduncle is 3–3½ cm. in diameter at the point where it enters the base of the placentome (the peduncular fossa). That part of the placentome adjacent to the point of attachment of the peduncle is turned under all round its circumference so that, except at the peduncular fossa, the outer aspect of the placentome lies against the uterine wall (see Text-fig. 3).

The membranous chorion is continuous with the cotyledon close to the peduncular fossa. The chorionic epithelium does not appear to be in close contact with the maternal epithelium at the base of the peduncle. Some 2–3 cm. from the base of the peduncle the chorion is separated therefrom by a varying number of large, loosely arranged, plump villi which project some way into the space formed at the edge of the peduncle (Text-fig. 4). The maternal epithelium is intact around the base of the peduncle and passes up the sides of the peduncle, where it is thrown into a number of folds, to be continuous with the epithelium lining the crypts of the caruncle. The maternal epithelium in this region is pseudo-stratified and columnar.



Text-fig. 4. A diagram of a segment removed from a placentome of *Elaphurus davidianus*, showing the relationship of the membranes and the chorionic villi to the caruncle. The top right hand of the drawing indicates how the villi project into the crypts of the caruncle.

Each placentome is covered on its foetal surface by allantois which is reflected on to the chorion from the edge of the placentome some distance from the attachment of the chorion at the peduncular fossa (Text-fig. 4). The allantois is a thin translucent membrane and strips readily from the placentome except at its point of attachment at the periphery. The chorio-allantoic vessels ramify on the surface of the cotyledon immediately outside the allantois.

While the above description is of the usual arrangement of the placentome, in a few instances there is some modification of the basic pattern. The cotyledon is occasionally found as a convex, ellipsoidal mass folded upon itself.

On sectioning the placentome, it is found to consist of a large number of chorionic villi projecting into the crypts of the maternal caruncle (Text-fig. 4). There are numbers of short, plump, common stems from which three to five secondary villi arise. The villi are distributed evenly over the cotyledon and each secondary villus projects into a crypt of the caruncle at right angles to the surface of the placentome (see Text-fig. 4). There does not appear to be any branching of the secondary villi after their origin, but a few small projections some 50–100 μ in length can occasionally be seen about the middle of a secondary villus.

The fact that there is an even distribution of the villi allows some computation of their number. In a placentome of length 10 cm., breadth 6 cm. and depth 4 cm., there are approximately 10,000–12,000 stem villi and 46,000–47,000 secondary villi. The total surface area of the villous bearing portion of the cotyledonary chorion has been estimated at 114–118 sq.cm. and there are about 100 stem villi and about 400 secondary villi arising from each sq.cm. The largest villi, including the stem portion, measure 12–14 mm. in length; the secondary villi, which are nearly all in contact with the maternal epithelium, measure 9–11 mm. in length and have a diameter of 0·5 mm. at their origin and 0·1 mm. at their tips. Each secondary villus can be considered as a truncated slender cone whose surface area is of the order of 10 sq.mm. The total surface area of the villi in one cotyledon is therefore of the order of 400,000–470,000 sq.mm. in a cotyledon of the size indicated.

In freshly injected, unfixed placentome, the villi may be readily extracted from the caruncular crypts (Pl. 3, fig. 8). There is much mucoid (Schiff positive) material present between the stem villi and also a thin discontinuous film of this material can be demonstrated between the epithelium of the villus and the crypt epithelium. The presence of this material enables the villi to be extracted from the crypts with the aid of dissecting needles.

The caruncular tissue does not reach to the surface of the placentome. The walls of the crypts end at approximately the level of the formation of the secondary villi. Numerous slender tapering threads of a fibrous nature, however, not covered with epithelium, and in which no blood-vessels have been seen, project from the walls of the crypts and interlace between the stem villi but do not separate all the stem villi from each other (Pl. 1, fig. 4). In placentomes from which all the foetal tissue has been removed, the maternal element is left in the form of an ovoid pedunculated mass, punctured by numerous deep crypts, from the surface of which the fine threads, described above, project like the pile on a carpet. It is difficult to see what function these threads perform and it is suggested that they are the remnants of the caruncle left after the invasion of the chorionic villi.

THE FOETAL BLOOD VESSELS

It has already been stated that one umbilical artery and vein pass close together direct to the two large cotyledons in the pregnant horn and that the other umbilical artery and vein pass into the non-pregnant horn to supply the three smaller cotyledons (Text-fig. 3). The main vessels bifurcate and send a branch to each large cotyledon. Each of these cotyledonary branches passes across the inner (foetal) aspect of the cotyledon lying immediately below the allantois (Pl. 1, fig. 1). The membrane can be easily stripped off the foetal vessels as they cross the surface of the cotyledon. The cotyledonary branches of the umbilical artery and vein give off, or receive, numerous large and small branches on the surface of the cotyledon. The main divisions usually fan out over the centre of the cotyledon, but there does not appear to be evidence of a distinct segmental pattern; subsidiary divisions of one main branch invade areas supplied by those from a neighbouring main branch and circumferential or peripheral branches are not uncommon. Arteries cross veins and vice versa with equal frequency.

The arterial branches to the stem villi leave the surface vessels at right angles to pass directly into the substance of the cotyledon (Pl. 1, fig. 3). The terminal branches of the cotyledonary vessels bend abruptly to penetrate the cotyledon. The veins from the stem villi either appear on the surface of the cotyledon and rapidly unite to form large surface vessels (Pl. 1, fig. 1), or else enter directly the under surface of the larger veins.

The umbilical artery and vein to the three smaller cotyledons in the non-pregnant horn pass close together round the lower edge of the septum. The artery gives off one large vessel to each cotyledon which ramifies over the surface, dividing repeatedly as in the other cotyledons. Smaller arteries are also occasionally given off from the main umbilical vessel and pass peripherally round the edge of the cotyledon. The branches of the umbilical vein follow a similar course.

The injection of one umbilical artery resulted in the rapid filling of the other artery through the numerous anastomotic vessels in the allanto-chorion. Numerous small vessels leave the umbilical artery and ramify in the extra-cotyledonary allanto-chorion and in the small portion of the amnio-chorion related to the anti-mesometric border. Further small vessels pass into the extra-cotyledonary allanto-chorion from the cotyledonary vessels. No arterio-venous anastomoses have been found in the cotyledons or in the extra-cotyledonary areas. In one specimen, however, quite large arterio-venous anastomoses were found between the large branches of the umbilical vein and artery. The distribution of the extra-cotyledonary vessels will be described later.

THE HISTOLOGY OF THE PLACENTOMES

The structure of the placentomes is not uniform throughout; the proportion of foetal and maternal elements varies and three distinct zones are present (Pl. 1, fig. 2). The inner (foetal) zone contains the stem villi arising from the allanto-chorion; a narrow intermediate zone is present at the point of division of the stem villi and includes a small portion of the caruncle; an outer (uterine) zone is composed of the secondary villi and the remainder of the maternal caruncle (Text-fig. 4). Each stem villus contains a core of pale-staining embryonic mucoid connective tissue with widely separated stellate cells. Branches of the chorionic vessels pass through the centre of a stem villus and give branches to the secondary villi. It is difficult histologically to differentiate arteries from veins, owing to the almost equal thickness of their coats, although the arteries have usually the thicker wall. Neither arteries nor veins possess elastic tissue in this situation. The stouter stem villi are usually covered with a high columnar epithelium (the cells being 30–35 μ in height) which may be pseudo-stratified. The epithelium at the bases of the stem villi is regular, but towards the point of bifurcation it is sometimes markedly folded, giving rise to a series of crypts about 60 μ deep separated by folds equally high. The epithelial cells stain very palely, and the single nucleus is usually placed in the basal portion of the cell (Pl. 1, fig. 4). These cells contain considerable quantities of glycogen and muco-polysaccharides and they are probably the source of the mucoid material which is so abundant round the stem villi.

The blood supply consists of a few small capillaries arising from the stem vessels which run to the area immediately below the basement membrane and are continuous with tributaries of the vein. There are, however, no intra-epithelial capillaries, binucleated or 'giant' cells in the epithelium in this situation.

One to five (usually two to three) secondary villi arise from each stem villus in the intermediate zone. The first half millimetre of each secondary villus has the same structure as the stem villus, except that the diameter of the villus is now much reduced. The character of the epithelium changes abruptly (Pl. 3, figs. 9–11), and the single-layered columnar epithelium is replaced by a more complex one. It consists essentially of a layer of cytotrophoblastic cells, one, two or even three layers thick. These cells are generally cuboidal or low columnar, and they possess a brush border where they lie on the surface (Pl. 4, fig. 13). The peripheral part of the cytoplasm is highly acidophil and granular, particularly at the area of contact with the maternal epithelium, whereas the inner part is palely basophil, granular and frequently contains vacuoles. Besides these typical cytotrophoblastic cells, large numbers of 'giant' cells are present (Pl. 4, fig. 12). These are large spherical or polyhedral cells and possess from one to four characteristic large nuclei. The nuclei have no nucleolus, and their chromatic material is arranged in numbers of discrete large and small granules. These stain intensely with all nuclear stains and particularly brightly with the Feulgen reaction. Some nuclei frequently stain so intensely as to appear as pyknotic masses (Pl. 4, fig. 17). The cytoplasm of these 'giant' cells is granular, basophil and often vacuolated, and also frequently contains Schiff-positive material, particularly in the larger cells and those which have heavily staining nuclei and vacuoles. The Schiff positive material is not always directed towards the maternal epithelium (see Wimsatt, 1950, p. 408), but is particularly pronounced in the juxta-nuclear cytoplasm and about the vacuoles when the latter are present.

The position of these 'giant' binucleated cells in the foetal epithelium varies. They are distributed equally in the inner aspect, within, and on the surface of the villous epithelium. The cells lying on the surface do not possess a brush border. The position of these binucleated cells shows very clearly, under the phase-contrast microscope, which also emphasizes the presence of vacuoles, nuclear structure and absence of brush border. The migration and fate of these cells will be described in a later section. The foetal epithelium in this intermediate zone is characterized by the presence of intra-epithelial capillaries (Pl. 5, figs. 18, 19).

The arrangement of the umbilical arteries and veins and their branches to the cotyledons is described on p. 212. The blood supply of the villi was examined by a variety of methods (see p. 204). Branches pass from the undersurface of the allanto-chorionic vessels as the latter cross the surface of the cotyledon and each divides into several subsidiary branches which pass into the centre of each stem villus. Within the stem villi, each artery divides to send arteriolar branches into each secondary villus (Pl. 1, fig. 3). These branches pass down the centre of the secondary villus and lie fairly close to the main venous channel of the villus, separated only by a small amount of connective tissue. As already indicated, it is impossible histologically to tell arteries from veins in the villi from their structure, and use has to be made of injection techniques.

Immediately peripheral to the point of transition of the two types of epithelium covering the villi, numerous small rapidly branching capillary twigs arise from the arterioles. These capillaries pass radially towards the epithelium, fanning out up and down the villus. The twigs arise from the arteries at irregular intervals of 0·25 to 0·5 mm., and in general they are directed to the nearest portion of the epithelium, that is, away from the central vein. On reaching the epithelium, these twigs anastomose with an intra-epithelial capillary network which runs throughout the entire epithelium of the secondary villus in the form of a network resembling wire-netting when seen under the binocular dissecting microscope (Pl. 1, fig. 3). The longest segments of the capillary network are directed mainly circumferentially round the villus, the shorter segments run either vertically or obliquely to anastomose with the longer segments. The mesh-work of capillaries so formed encloses groups of six to ten cytotrophoblastic cells. There is no doubt that the network of capillaries is continuous over the whole of the secondary villus, except its basal portion, and that it possesses a distinct topographical pattern.

The artery continues to the tip of the villus, frequently without branching, but sometimes a second smaller artery arises high up the secondary villus and runs parallel to the first. The artery terminates in a series of twigs similar to those arising laterally higher up. There does not appear to be a terminal arterio-venous loop.

The main venule runs parallel to the main arteriole in the centre of each secondary villus. In its course, it is joined by numerous capillaries which appear to be longer and narrower than the corresponding ones arising from the arteriole. There are frequently long slender venous tributaries, receiving several series of collecting venules, which enter the main vein some distance higher up the villus. In all the injected specimens, it is remarkable how constantly injection of the arterial or venous side of the system, both with controlled pressure injection or manually from a syringe, results in the even filling of one half of the capillary network. In all the well-injected villi, that half of the capillary network nearest the artery contains red dye which is replaced by blue dye in the other half. It would appear that the main vessel is filled first, and that the injection mass then runs almost equally into all the lateral twigs and so into the capillary network. The direction of the blood-flow is therefore probably in a horizontal direction circumferentially about the villus, except that at the origin of the secondary villus, where the flow may be to some extent towards the tip of the villus owing to the relatively more peripheral origin of the collecting venules in many villi.

Histological examination confirmed the arrangement and distribution of these vessels and their connexions with the intra-epithelial capillaries (Pl. 5, figs. 18, 19). The capillaries, which are almost exclusively within the foetal epithelium, are found to penetrate it to a varying extent. At *no point* has a capillary been observed with its endothelium presenting on the surface of the villus. The endothelium of the capillary is *always* covered by part of an intervening trophoblastic cell. Examination with the phase-contrast microscope reveals that the capillaries are formed of tubes of endothelium, the flattened nuclei of the cells being clearly seen, but there is always a covering flange of trophoblastic cytoplasm at points where the capillaries reach nearest to the surface (Pl. 5, fig. 19). Silver staining methods for the demonstration of reticulum show the presence of sparsely distributed fine fibrils about the foetal capillaries.

The villi narrow considerably towards their tips, the epithelium covering the villus remains several cells thick, and there is much less mucous connective tissue. The main vessels have not decreased in diameter in proportion to the narrowing of the villus, and still remain quite large. The main difference in the area of the cotyledon in which the tips of the villi are found is in the maternal elements, which will now be described.

The maternal part of the placentome is derived from the caruncular portion of the uterine mucosa. In the four specimens each placentome is raised on a pedunculated mass attached to the wall of the uterus. The peduncle is elliptical in cross-section, and is covered by pseudo-stratified maternal epithelium. It contains numbers of uterine glands embedded in a dense mass of fibrous tissue which composes the body of the peduncle. Through it pass the main vessels supplying the placentome.

At the point of junction with the main mass of the placentome, the peduncle is everted and widens considerably to form the basal part of the placentome (Pl. 1, fig. 2). On this everted portion, uterine glands are still present. The dense connective tissue of the base of the peduncle is now replaced by a zone of more loosely arranged connective tissue in which the large maternal blood vessels ramify. This, in its turn, is replaced by a further zone, 1 mm. thick in the largest placentome, consisting of densely packed connective tissue fibres whose main direction is parallel to the long axis of the placentome. The maternal vessels send their branches to the caruncular septa through this layer, and the tips of the foetal villi extend as far as its superficial zone. The remainder of the caruncular portion of the placentome consists of three distinct zones as described for the villi (Pl. 1, fig. 2), alike in the fact that they form the walls of the crypts containing the foetal villi, but differing in their histological structure. The peripheral zone, nearest the uterine wall, is the most extensive, being 6–8 mm. in depth in the largest placentomes. It contains the major part of each of the secondary villi. In this zone the crypts are all lined by a maternal epithelium which is not a syncytium, but consists of distinct cuboidal cells with definite cell membranes. This epithelium rests on a basement membrane, and at the bottom of the crypts it tends to be two to three cell layers thick. The supporting tissue of the walls of the crypts consists mainly of reticular tissue with few collagenous fibres. At the points of junction of several separate walls of caruncular tissue there is more collagenous tissue and large vessels can frequently be seen (Pl. 3, fig. 10). Essentially the caruncle consists of a series of vertically directed, relatively thick, columns, containing collagenous fibres and the larger vessels, which are interconnected by thin vertical septa forming a network surrounding the crypts. The main maternal blood vessels are not regularly distributed in the caruncle but are found at intervals throughout the placentome. Seldom are the main maternal veins or arteries found adjacent to one another. The main maternal arteries pass upwards in the thicker vertical columns, occasionally give rise to large branches, and give off fairly regular short lateral branches, which either divide at once into numerous capillaries, or pass up the edge of a septum for a short distance, giving off a series of laterally directed capillaries. These capillaries fan out laterally and join a vertical capillary network, which traverses the whole extent of the walls of each crypt. The main direction of the capillary network is horizontal—that is, passing tangentially at right angles to the long axis of the foetal villus. The capillary network has a distinct uniform pattern, but its meshes are somewhat shorter and are more equal in length than those of the foetal capillary network. This maternal capillary network runs for the most part immediately beneath the basement membrane supporting the maternal epithelium. The capillaries are never 'intra-epithelial', as in the villi, but are always covered by a few reticular fibres, the basement membrane and the maternal epithelium. The capillary network is drained by a number of efferent capillaries, which either collect into larger tributaries or drain directly into the main maternal veins.

It appears that the relationship of the foetal to the maternal circulation is that of one cylindrical capillary network within another. The blood circulating in the two networks is always separated in this region by at least the foetal endothelium, portions of a cytotrophoblastic cell and a maternal epithelial cell and the maternal endothelium. It is difficult to estimate the distance between the two circulations owing to the separation of the walls of the villi from those of the crypt at fixation. However, the least distance observed in sections is $8\,\mu$, the most usual distance is from 10 to $12\,\mu$, and occasionally the two circulations are separated by tissues up to $25\,\mu$ thick. It is very probable that, in life, the distances are even less.

The intermediate narrower zone of the placentome, which is only about 1–2 mm. in depth, is fundamentally similar, except for the presence of numbers of large multinucleated cells, adjacent to or intercalated in the maternal epithelium (Pl. 3, fig. 11; Pl. 4, fig. 16). The cells, however, are never overlapped by a layer of maternal epithelium, nor do they invade the underlying stroma.

These multinucleated cells vary considerably in their size, in the number of their nuclei and in their histochemical reactions. All variations from small cells with two or three nuclei to the largest syncytial masses, with over twenty nuclei and measuring as much as $150\,\mu$ across, have been observed (Pl. 4, fig. 17). The nuclei, which are devoid of a nucleolus, are spherical, densely staining and their chromatin is arranged in the form of numerous small spherical granules. It is significant that the binucleated foetal cells exactly resemble the multinucleated cells found within the maternal epithelium. Staining with the Schiff reagent and examination with phase contrast reveals these cells in every position from the inner aspect of the foetal epithelium to a site intermediate between the two epithelial layers and finally intercalated among the maternal epithelial cells (Pl. 4, figs. 14–16). Mitotic figures have not been observed, but it is probable that the increase in the number of nuclei is brought about by nuclear division and not by fusion of neighbouring cells. The cytoplasm does not always possess Schiff-positive material; the quantity of this material appears, in each cell, inversely proportional to the number of nuclei. The cytoplasm of many of the cells is heavily vacuolated (Pl. 4, fig. 16), and it has been possible to demonstrate the presence of small quantities of alkaline phosphatase in some of the cells.

The third or innermost zone of the placentome contains the stem villi and the thin avascular processes of a fibrous nature already described (Pl. 1, fig. 4). The transition is abrupt; at one level the caruncular septa are as described above, with multinucleate cells, maternal epithelium, maternal vessels and a connective tissue core, but it is immediately replaced by an avascular fibrous strand only some 10–20μ thick. The part of this strand in continuity with the intermediate zone contains nuclei of fibrocytes, but a short distance away they become pyknotic and, for the terminal 2 mm., the strands consist only of collagenous material, which occasionally gives the appearance of breaking apart into interlacing strands of amorphous material.

THE STRUCTURE OF THE MEMBRANOUS CHORION

The membranous chorion is found in the intercotyledonary area of the placenta and is held against the uterine wall by the pressure of the amniotic and allantoic fluids. In all four specimens the membranous chorion separated easily from the uterine wall, and as a result it was difficult to cut sections with the chorion remaining in contact. In the great majority of sections taken through different areas where the membranous chorion remained in contact with the uterine wall the uterine epithelium was present. The uterine epithelium consists of either a flattened single-celled layer, or, particularly in relation to the areolae, a pseudo-stratified or stratified columnar layer of cells. It was missing in sections in many places, but it was impossible to decide whether this was due to its original absence (as a definite entity), which implies its attrition by the chorion, or whether it had been lost during fixation, or whether separation of the chorion caused its avulsion. However, the uterine epthelium was present in all the areas examined where the chorion remained in contact with the uterine mucosa. This does not mean that there may not have been earlier epithelial destruction with subsequent regeneration of the epithelium, as occurs in the sheep.

The membranous chorion is avillous and is a single layer of trophoblastic columnar cells, sometimes with a pseudo-stratified appearance, which contains numbers of binuclear giant cells. However, at regular intervals the membranous chorion is thrown into a series of folds which consist when examined from the outer aspect of numbers of closely packed short folds or pleats with a mesenchymal core, and which are frequently folded back on themselves (Pl. 5, fig. 20), so that in histological preparations a tip of a fold may be seen in cross-section (Pl. 5, fig. 21). So far as can be ascertained the areolae are found in relation or in proximity to the mouths of the uterine glands. In all the specimens the areolae varied in size from 1 to 3 mm. in diameter, the majority being 2–3 mm. across.

The columnar trophoblastic cells are tallest (45–60μ) in the crypts between the folds, and each columnar cell possesses what appear to be projections of cytoplasm from its free surface. These projections can be seen within the thin layer of what is presumably secretory material which everywhere lies between the uterine epithelium and the trophoblast. The columnar cells are frequently vacuolated, the cytoplasm is acidophilic, and the nuclei are large and vesicular. The vacuoles are usually in the distal portion of the cell, and show no evidence of terminal bars. Little basophil material or Schiff-positive substances are present in the columnar cells and no glycogen could be demonstrated. The binucleated cells appear to be distributed evenly throughout the areola and in the same number as is found in the inter-areolar membranous chorion. The binucleated cells are essentially similar in their cytological characteristics to those found in the cotyledon.

No intra-epithelial capillaries have been seen anywhere in the uterine epithelium or on the trophoblastic epithelium of the membranous chorion. The maternal capillaries lie immediately adjacent to the maternal epithelium, forming a marked subepithelial capillary network. The foetal capillaries are also placed immediately below the trophoblast, but none have been seen either invading or within the trophoblast itself, as has been found in the trophoblast of the cotyledon. The vessels of the membranous chorion are either derived directly from the umbilical (allantoic) vessels or from vessels which, having pursued a course over the cotyledon and having sent tributaries into it, continue over the edge of the cotyledon and run into the membranous allanto-chorion. The areolae obtain a more copious blood-supply that the inter-areolar region and each areola has an extensive capillary plexus. The arterial branches usually divide dichotomously, but numerous slender collaterals are given off along the course of the main and subsidiary vessels. The slender terminal branches of the arteries usually pass one to each areola, whereas two or more adjacent areolae are drained by slender, short channels which enter a single venule. There is evidence from one of the injected specimens of the existence of a collateral circulation from one cotyledon to another through the vessels of the allanto-chorion.

DISCUSSION

Andresen (1927) has reviewed the literature on the placentation of a number of Cervidae and gives a table indicating the number of placentomes present in the various species. We have already pointed out (Hamilton & Harrison, 1951) that the placentomes in *Elaphurus* are fewer in number than in most species yet described. In our pregnant specimens there are five or six placentomes, and five caruncles are present in each non-pregnant uterus. Turner (1878) observed five cotyledons in *Cervus* (*Hyelephas*) *porcinus*. Boulart (1888) states that there are six large and eight small cotyledons in *C. porcinus*. He also states that there are five or six cotyledons in *Capreolus capreolus* and six to seven in *Cervus rufus*. Andresen (1922), however, found the number of cotyledons in *C. rufus* varied from six to nine. Turner (1878) observed six cotyledons in *Rangifer tarandus*, as also did Beauregard & Boulart (1885) and Kolster (1909). In *Rucervus eldi* (*Panolia frontalis*), Beauregard & Boulart (1885) found nine cotyledons; these authors also found six cotyledons in *Cervus* (*Sika*) *nippon*.

When comparison is made with the cotyledons of the placenta of the Bovidae, the Capridae, the Antilopidae and the Giraffidae it is apparent that, although there are few cotyledons in *Elaphurus* and in some other species of Cervidae, the number or size of the cotyledons is of little value in the classification of the Cervinae.

The form of the cotyledons in the groups mentioned above also shows considerable variation. Andresen (1927) has described three main types in the Artiodactyla; they are a flattened type which is characteristic of some Antilopidae; a convex type found in Bovidae, Cervidae and other Antilopidae and a concave type as in the Caprinae, the Capridae and the Rupicaprinae. The form of the cotyledon in *Elaphurus* is convex and pedunculated and bears a striking resemblance to that found in *Cervus elephas* (*rufus*) (Andresen, 1922) and also to the shape of the cotyledon in *Rangifer tarandus* (Kolster, 1909) and as found by us in *Cervus nippon*.

It is apparent that the main cotyledonary masses develop in relation to the pre-existing caruncular areas and as already stated these are found linearly arranged along the dorso-lateral wall of the uterine horns. In one of our specimens of *Elaphurus* and in several species of Cervidae described by the authors mentioned above, and in other deer examined by ourselves, there have been small accessory cotyledons which develop mainly in relation to the septum. It is possible that these accessory cotyledons are either detached portions of a main cotyledon or are arising *de novo*.

Chorionic sac. The chorionic sac in *Elaphurus* entirely fills both horns of the uterus and in this respect is similar to other Cervinae. The amnion, however, does not line the whole of the chorion as described by Ray (Wood Jones, 1951), but is found mainly in one horn. A large allantois lies mainly in the non-pregnant horn and separates the amnion from the chorion (see Mossman, 1937, for a description of the membranes of *Cervus mexicanus*, plate 23). There are no villi on the foetal surface of the amnion, as Ray (Wood Jones, 1951) states, but there are numerous raised plaques or papilliform projections consisting of squamous epithelial cells. The latter are well known and occur in most Artiodactyla.

The chorion of the cotyledon of *Elaphurus* is villous; the villi consist of three to four long, filiform, cylindrical processes arising from a short, stout common stem. Occasional short lateral branches or short terminal bifurcations are found. The villi of the cotyledon of other Cervidae are either arranged in tufts, with numerous branches (*Rangifer tarandus*, Turner, 1878; *Cervus mexicanus*, Turner, 1879) or extend from a short stout base in the form of elongated filaments (*C. procinus*, Turner 1878; and *C. elephas*, Strahl, 1906). In the intercotyledonary area we find in *Elaphurus* numerous areolae similar in appearance to those described by Wimsatt (1950) in the sheep. It is almost certain that the polygonal areas of ridge-like elevations seen in the membranous chorion of *Rangifer tarandus* by Turner (1878) and the elevated vascular patches observed in the intercotyledonary area in *Hydropotes inermis* by Ewart (1878) are similar structures. Our material unfortunately does not permit us to give a detailed account of the development of the cotyledons or the areolae.

Placentome. Histological examination of the placentome shows, as already indicated, that it can be subdivided into three main zones in which the structure of the maternal and foetal tissue varies. This subdivision has already been noted by Strahl (1906) in *Cervus elephas*, by Kolster (1909) in *Rangifer tarandus* and by Andresen (1927) in *Cervus elephas*, and in other Cervinae. We confirm similar differences in the histological structure of the placentome and presumably in the function of the three zones. Histochemical investigations have shown that the basal part of the villus functions as a store for glycogen, the narrow intermediate zone contains numerous giant cells, and has invasive properties, whereas the peripheral and longest portion of the villus is constructed to permit physiological exchange. As will be discussed later, these suggested differences in function are confirmed by the arrangement of the blood-vessels. There is a similar difference in the histological structure and function of the caruncular portion of the placentome. The basal zone is constructed for physiological exchange, the intermediate zone shows evidence of attrition, and the superficial zone consists only of strands of almost avascular, and frequently necrotic, connective tissue. In spite of the attrition of the epithelium in the intermediate zone, there is no extravasation of blood from maternal vessels. Similar appearances have been found by Strahl (1906) and Andresen (1922) in *Cervus elephas* and by Kolster (1909) in *Rangifer tarandus*.

Giant cells. There are two conflicting opinions concerning the origin of the giant cells of the intermediate zone. Strahl (1906, 1911) was of the opinion that in *Cervus elephas* these cells arose from maternal epithelium. On the other hand, Assheton (1906), Kolster (1909), and Andresen (1922) regarded the cells as foetal in origin. Recently, Wimsatt (1950, 1951), using newer histochemical techniques, confirmed the foetal origin of the giant or syncytial cells in the sheep, having earlier held the contrary view (1949). Using similar techniques, our findings agree with those of Wimsatt as to the origin of these cells in *Elaphurus*. They pass through the trophoblast in the invasive zone to lie against and eventually become intercalated in the maternal epithelium. This passage of the giant cells through the trophoblast and into the maternal epithelium does not appear to occur in the outer zone of the placentome or in the areolar regions, although binucleated giant cells are commonly found within the trophoblast in these zones. There is evidence that many of the giant cells which invade the maternal epithelium are eventually destroyed and it is probable that the invasion is a continuous process and is associated with the growth of the placentome.

Blood-vessels. As in the placenta of many ruminants, a single artery enters the base of each stem villus and sends a branch to each secondary villus, which runs down the centre of the villus accompanied by a vein. At the tip of the villus the artery divides into a terminal leash of capillaries which drain back into the vein. At irregular intervals down the secondary villus numerous lateral branches arise from the central artery and pass to the circumference of the villus to enter the capillary plexus which lies within the trophoblastic epithelium. This is essentially similar to the vascular arrangements described by Wimsatt (1950) in the sheep. As first noted by Tafani (1886) in the pig, and since by a number of other investigators in many ruminants, the capillaries form an extensive capillary intra-epithelial network which in *Elaphurus* has an almost topographical pattern. We find—as also did Tafani (1886), Jenkinson (1906) and Wimsatt (1950)—that the capillaries are everywhere covered with thin layers of trophoblast, which is in contradistinction to the view of Barcroft & Barron in the sheep (1946). We find an equivalent sub-epithelial network of capillaries extending throughout the maternal septa. As well as the profuse blood-supply to the cotyledon there is an excellent supply to the membranous chorion, and a marked sub-epithelial capillary network is present in relation to the areolae. As has been noted, the latter are found in relation to the mouths of the uterine glands; and it must be added that in none of our sections have we observed definite evidence of the destruction of maternal epithelium. However, our specimens are relatively late in pregnancy and it cannot be stated whether there is any destruction of the uterine epithelium at an earlier stage, as occurs in the sheep during the early stages of pregnancy (Wimsatt, 1950; Amoroso, 1951). Several previous investigators (Kolster, 1909; Andresen, 1927; Brambel, 1933; Wimsatt, 1950) have discussed the possibly different functions of the cotyledonary, areolar, and inter-areolar regions of the placenta in the Artiodactyla. Our investigations on *Elaphurus*, when considered together with those of the above workers, and also those of Wislocki & Dempsey (1946), do indeed suggest that, in the later stages of pregnancy at least, the cotyledon is primarily concerned with the interchange between the maternal and foetal blood streams, whereas in the inter-cotyledonary area there is mainly an 'absorption' of material from the uterine glands and uterine epithelium. It is probable that the process of 'absorption' may involve complex histochemical activity by the trophoblast, and is not necessarily as simple as earlier workers believed

Classification. Since Grosser's original classification (1927) of the arrangment of the foetal and maternal tissues, attempts have been made to fit each newly described placenta into one or other category. It is now realized that this original classification cannot be applied too rigidly, since some reservation is usually necessary before placing the placenta of an animal in a particular category; such a *caveat* is particularly relevant in the description of the Ungulate placenta.

There is little doubt that, in the specimens we have described, an epithelio-chorial relationship prevails in the major part of the membranous chorion (see p. 218) and in most of the placentomes. In the narrow intermediate zone of the placentome, about the necks of the secondary villi, there is invasion of the maternal epithelium by foetal giant cells with some fusion of the foetal and maternal elements, which implies a syndesmo-chorial relationship. In the inner (foetal) part of the placentome, however, there has been complete destruction of the maternal epithelium and the chorion lies close to, but not in contact with, maternal connective tissue septa (see p. 218). Such septa seldom contain blood-vessels, and it is doubtful whether there is any physiological exchange occurring in this region. It is considered that such a relationship of foetal and maternal tissues could be described by the term *diaphthoro-epithelio-chorial.** This term is used by us to mean complete destruction of the maternal epithelium by the chorion, leaving the chorion in close proximity to, but *not fused* with, the underlying maternal stroma. It is probable that the appearance of such a relationship of the two elements of the placenta is associated in *Elaphurus* only with the growth of the maternal part of the placentome. It is, in fact, a reflexion of the reaction of the invasive trophoblast to the increase in size of the caruncular portion of the placentome during gestation. Were there not an invasive and attritional region at the base of the secondary villus it would inevitably be compressed by the tissue of the growing caruncle.

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* We are indebted to Prof. W. S. Maguinness of King's College, London, for his help with this term. διαφθορά means destruction, δια as a prefix expresses completeness.

SUMMARY

1. Descriptions are given of the gravid uterus, placenta and foetal membranes of four specimens of Père David's deer (*Elaphurus davidianus*) and of the genital tract of two non-pregnant adults and two female foetuses.

2. The chorionic sac occupies the entire uterine cavity, the amnion lies in the pregnant horn, and is separated from the chorion in the non-pregnant horn and partially in the pregnant horn by a large allantois.

3. The placenta is characterized by the small number of the cotyledons or placentomes (usually five), by their large size and by their convex shape.

4. The foetal villi of the cotyledon consist of long filiform processes, three or four of which arise from a common stem villus. The secondary villi seldom branch, and each enters a separate crypt in the caruncle.

5. The placentome exhibits three zones, each having a different structure and presumably different function.

6. The blood-supply of each villus consists of a central artery which sends numerous collateral branches to a well-defined retiform intra-epithelial capillary plexus; from this plexus collecting channels drain into the central vein.

7. The membranous chorion is avillous, but is folded upon itself in numerous places to form well-vascularized areolae which lie in relation to the mouths of the uterine glands.

8. The trophoblast contains numerous binucleated giant cells which in the intermediate zone of the placentome invade the maternal epithelium and bring about its complete destruction in the inner zone of the placentome.

9. The term diaphthoro-epithelio-chorial is suggested for the relationship of foetal and maternal tissues where the maternal epithelium is completely destroyed by the chorion and where the chorion lies close to, but not necessarily in contact with, the maternal stroma.

10. The placenta of the *Elaphurus* is mainly epithelio-chorial with syndesmochorial and diaphthoro-epithelio-chorial parts in the placentomes.

We are indebted to his Grace, the Duke of Bedford, for his kindness in presenting us with the specimens which are described in this paper; and to Mrs Osborne-Samuel for her help in arranging the collection of the specimens in a fresh state.

The technical work was carried out by Mr R. Watts, and the photographs were taken by Mr E. V. H. Pittock and Mr R. Q. Cox.

The expenses were partly defrayed by grants to us from the Central Research Fund, University of London.

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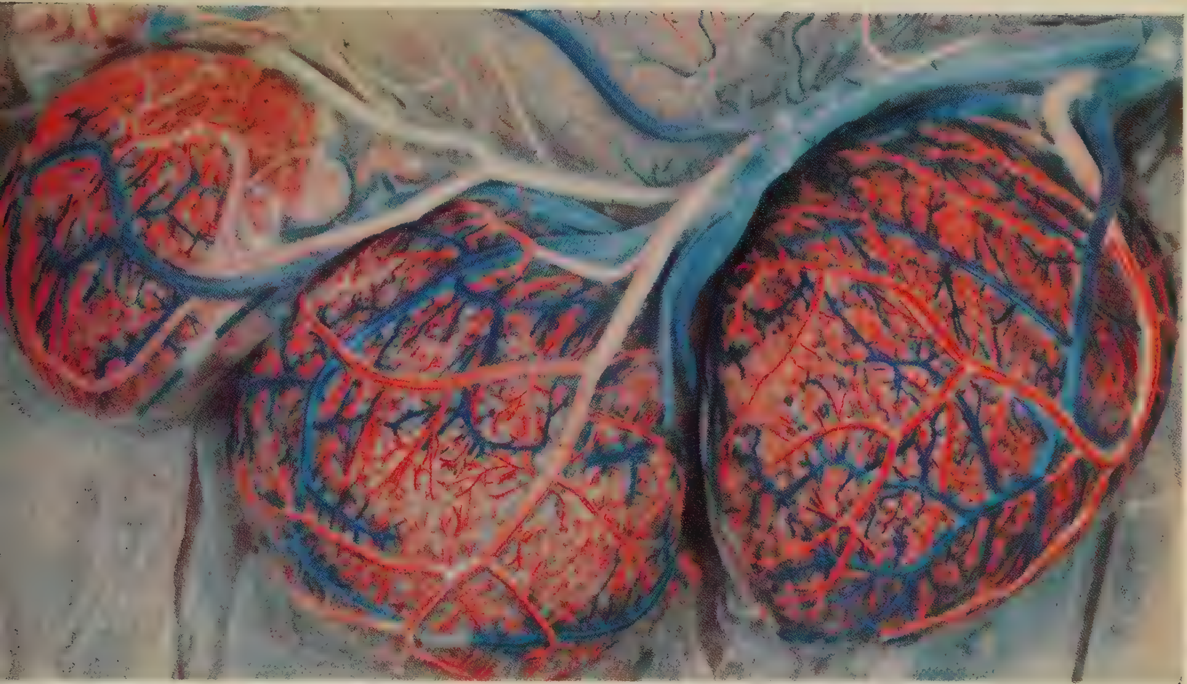

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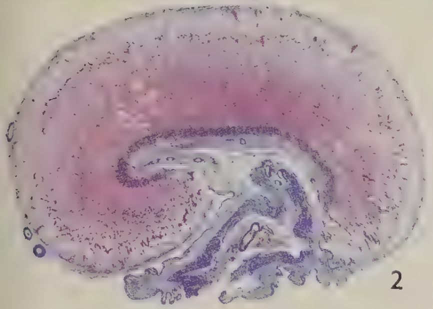

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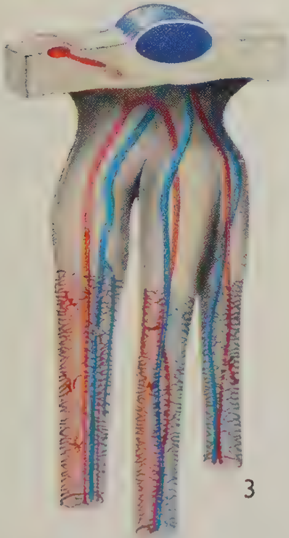

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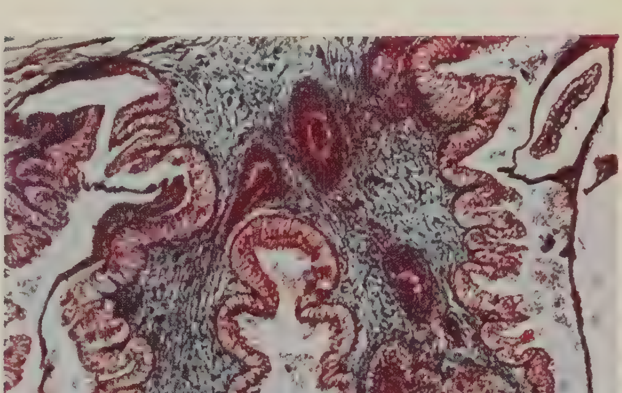

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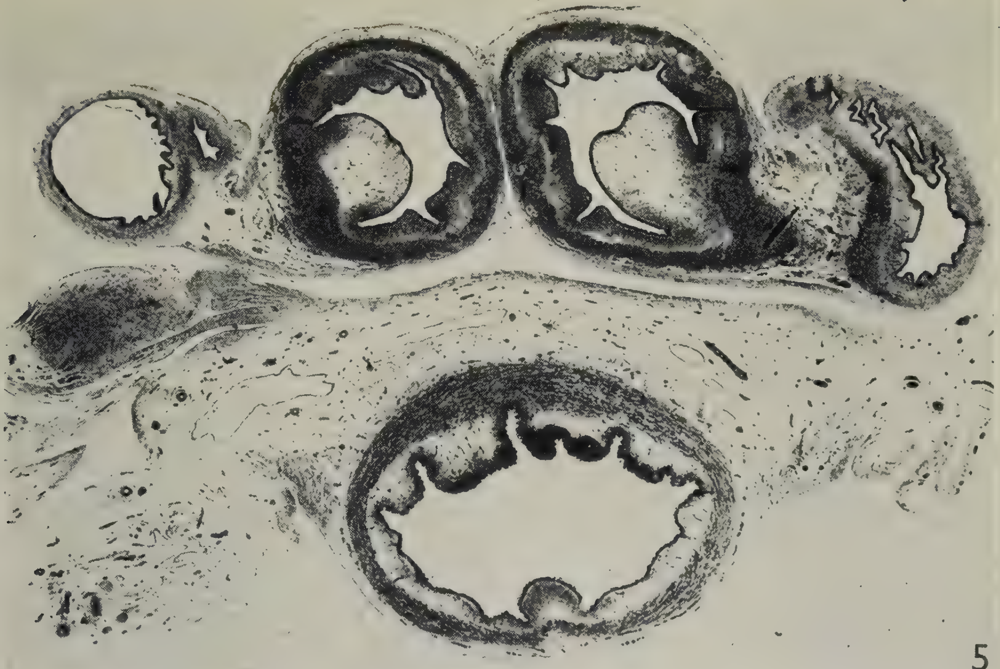

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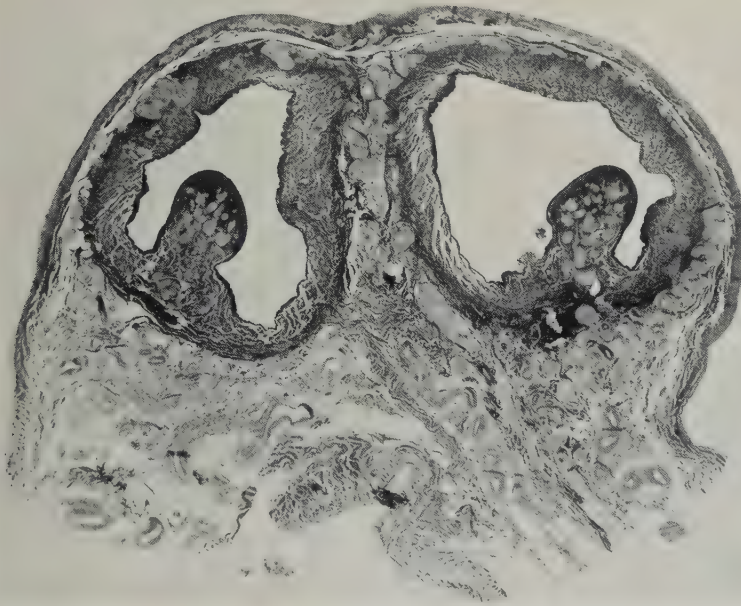

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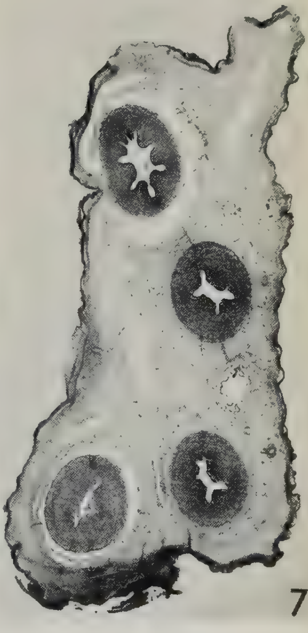

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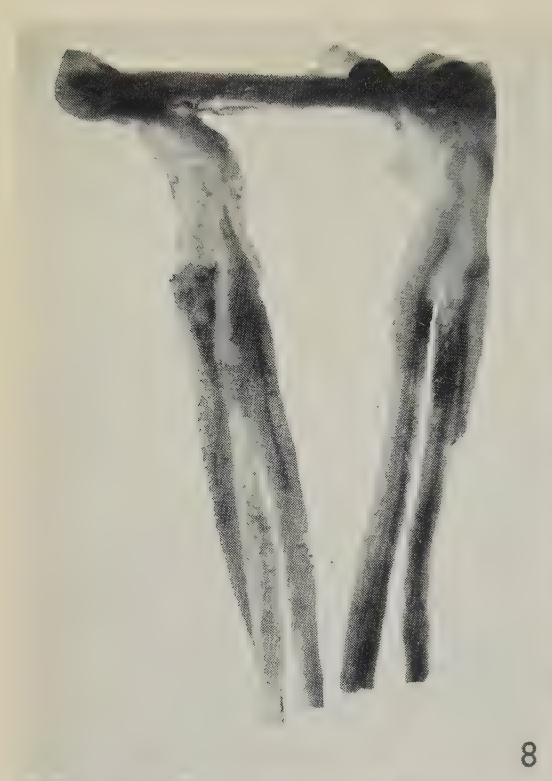
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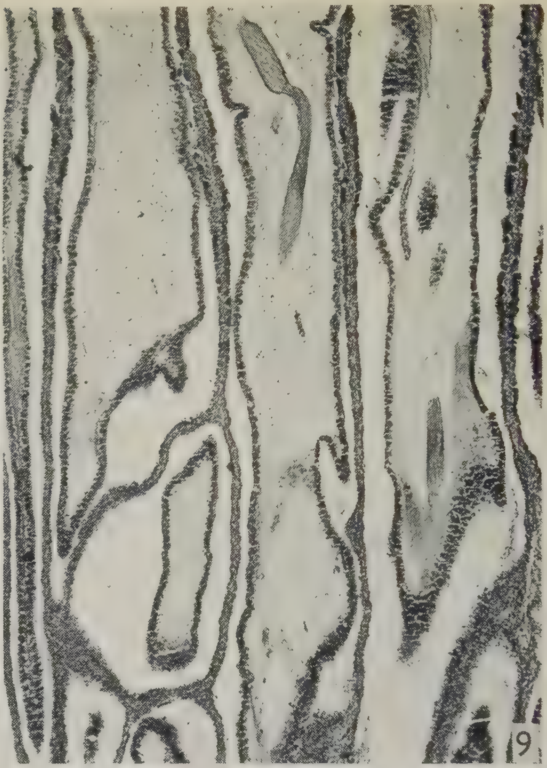
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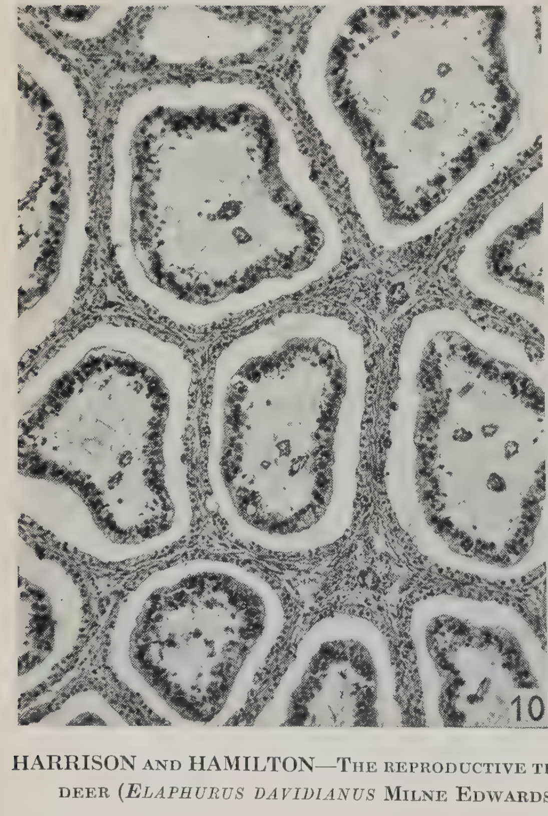
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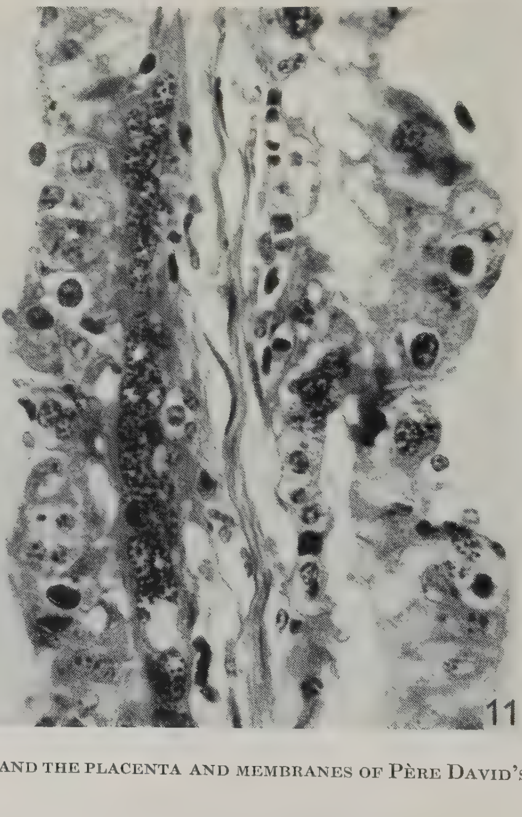
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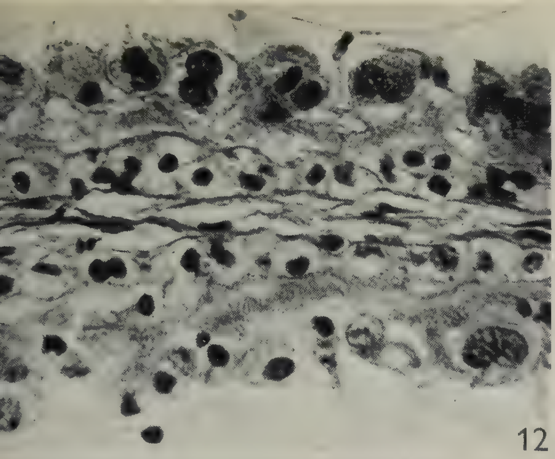
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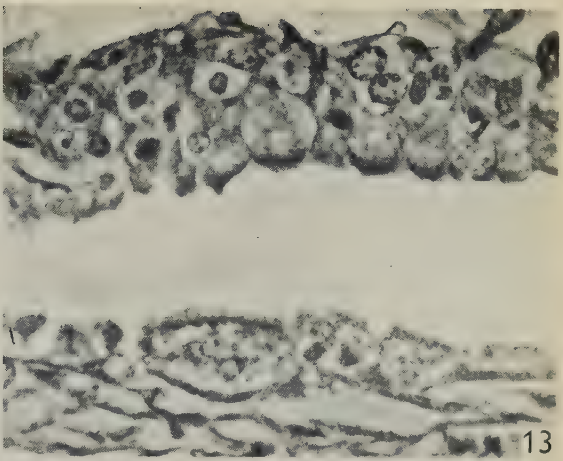
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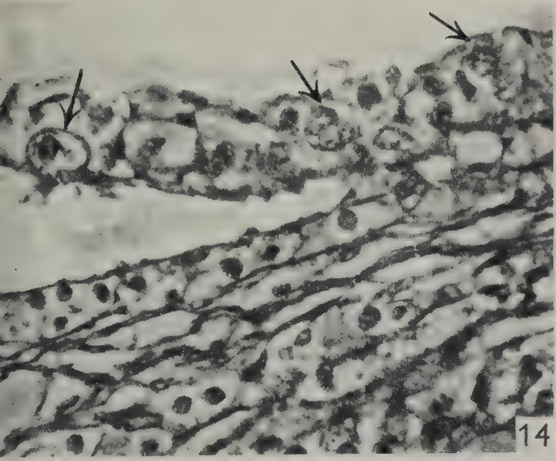
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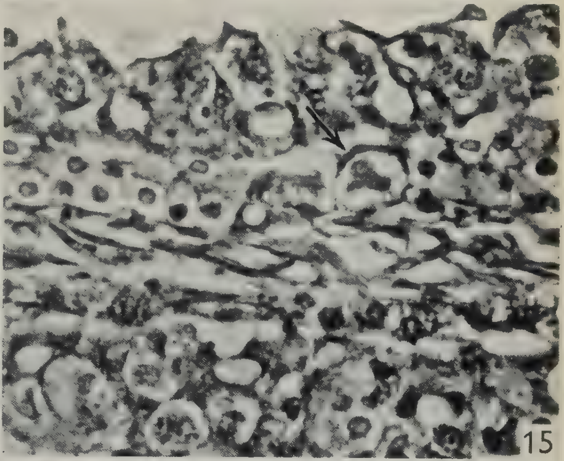
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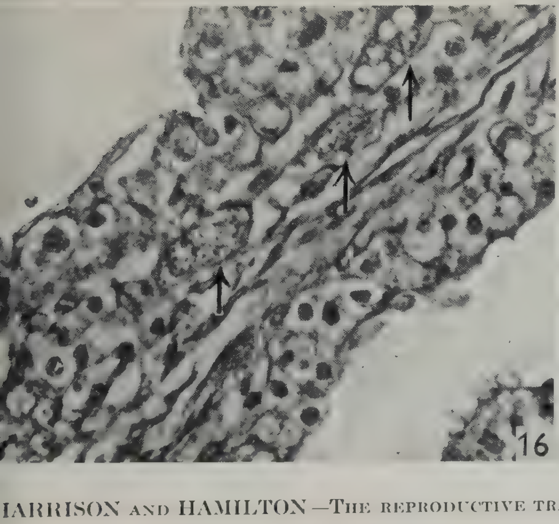
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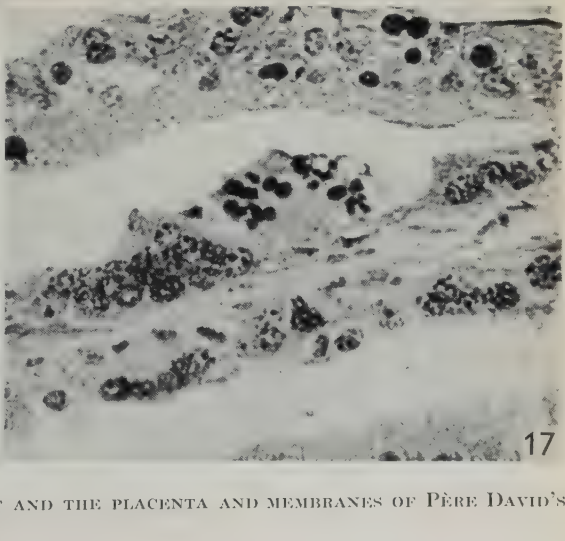
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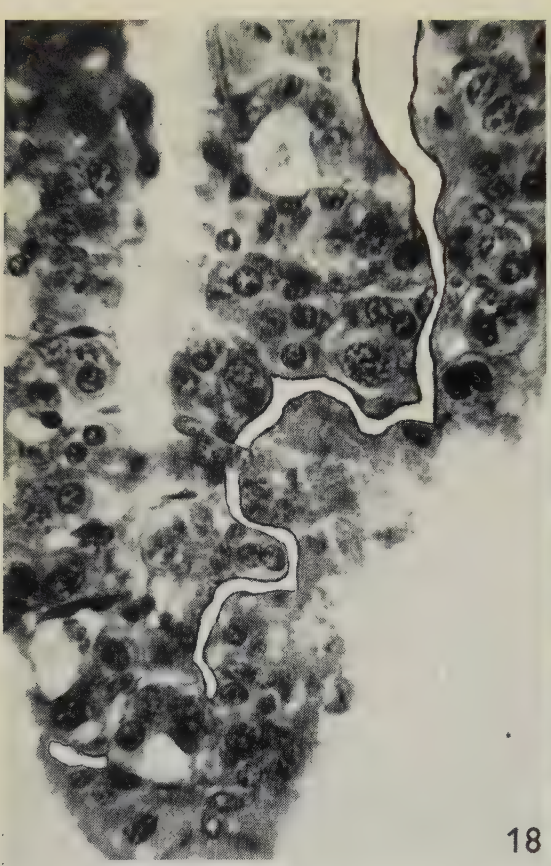
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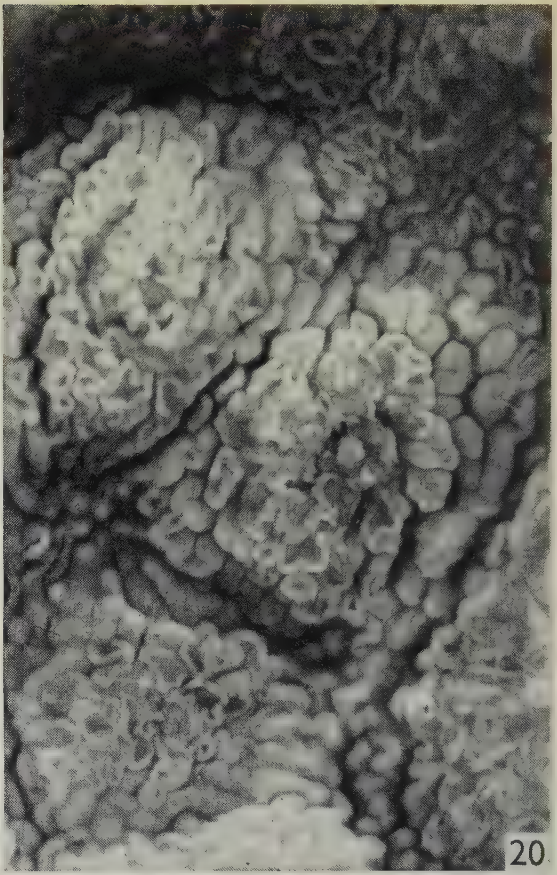
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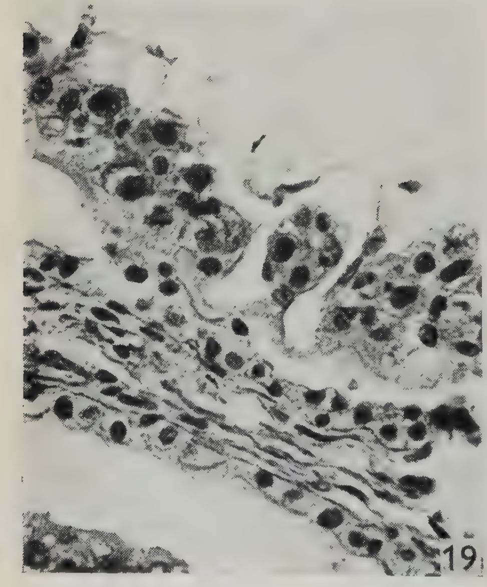
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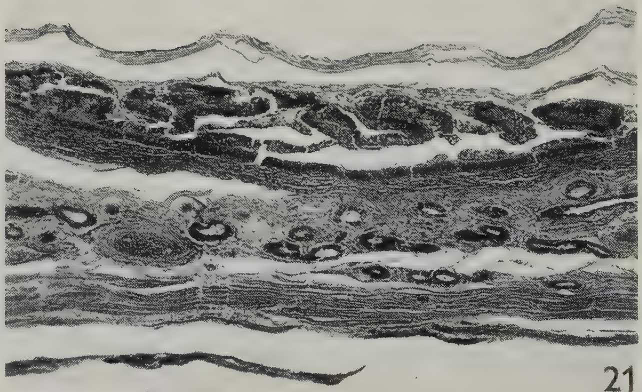
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HARRISON AND HAMILTON—THE REPRODUCTIVE TRACT AND THE PLACENTA AND MEMBRANES OF PÈRE DAVID'S DEER (*ELAPHURUS DAVIDIANUS* MILNE EDWARDS)

EXPLANATION OF PLATES

PLATE 1

Fig. 1. A photograph of three injected cotyledons in the left horn of a pregnant *Elaphurus davidianus* (PD 2). Nearly natural size.

Fig. 2. A photograph of a section through the middle of the placentome of *Elaphurus davidianus*. Stained by Masson's method. Natural size.

Fig. 3. A drawing of three injected villi which have been removed from the placentome. The central vessels of the villus and the intra-epithelial capillary network are shown.

Fig. 4. A photograph of a section through the inner (foetal) zone of the placentome showing the stem villus and its vessels, and on the left and right the thin strands of maternal connective tissue from which the epithelium has been completely removed. × 50.

PLATE 2

Fig. 5. A photograph of a coronal section through the vagina (below) and the uterine horn (above) of a 38 cm. foetus. × 6.

Fig. 6. A photograph of a coronal section through the fused uterine horns of an adult animal (PD 5). Note the caruncles and the numerous obliterated blood-vessels. × 2.

Fig. 7. A photograph of a cross-section of the umbilical cord. The allantoic duct can be seen on the right between the two arteries. Note the marked squamous plaque at the bottom left. × 2.

PLATE 3

Fig. 8. A low-power photograph of the villi after they have been removed from the crypts of the caruncle. × 10.

Fig. 9. A photograph of a section through the inner part of the placentome showing the plump stem villi and on the left a secondary villus. The maternal septa are clearly shown. × 20.

Fig. 10. A low-power photograph of a transverse section through the placentome showing the villi lying in the crypts of the caruncle. The villi have shrunk away from the maternal tissue due to fixation. The maternal epithelium is well shown. × 40.

Fig. 11. A photograph of a section showing the foetal epithelium on the left and right, and the maternal septum in the centre. A large multinucleated cell can be seen invading the maternal epithelium. × 300.

PLATE 4

Fig. 12. A photograph of a section showing the foetal epithelium (above and below), and the maternal septum in the middle. The large binucleated cells of the trophoblast are well shown and the maternal epithelium is intact. × 350.

Fig. 13. A photograph of a section using phase contrast to show the brush border on the trophoblastic epithelium. × 300.

Fig. 14. A photograph using phase contrast to show the position of binucleated cells as they pass through the trophoblast. × 300.

Fig. 15. A photograph using phase contrast to show a binucleated giant cell invading the maternal epithelium. × 300.

Fig. 16. A photograph using phase contrast showing the multinucleated cells, the top one with many vacuoles, intercalated in the maternal epithelium. × 350.

Fig. 17. A photograph of several giant cells which have invaded the maternal epithelium, and one of which shows a number of pyknotic nuclei. × 400.

PLATE 5

Fig. 18. A photograph of a secondary villus in which the course of an intra-epithelial capillary has been outlined. × 500.

Fig. 19. A photograph using phase contrast showing intra-epithelial capillaries with a thin layer of trophoblastic cytoplasm intervening between the capillary and the maternal epithelium. × 250.

Fig. 20. A low-power photograph of the outer aspect of the membranous chorion showing the folds of the areolae. × 20.

Fig. 21. A photograph of a section through an areola. From above downwards can be seen the allantois, the chorion, the intact uterine epithelium, the uterine mucosa and the uterine muscle. × 45.

IN MEMORIAM

THOMAS WALMSLEY, M.D., F.R.S.E., 1889–1951

Thomas Walmsley, Professor of Anatomy in The Queen's University of Belfast, died at his home in Armagh on 12 November 1951, after a trying illness, bravely borne. The son of an officer in the Royal Indian Navy, he was born in Bombay but was educated at Greenock High School and Glasgow University, in his ancestral Scotland. He graduated in Medicine in 1912 and was appointed Junior Demonstrator in Anatomy under Prof. T. H. Bryce. After two years in this post, he became House Surgeon to Sir William Macewen in the Western Infirmary of Glasgow and, at the same time, University Assistant in Surgery. Walmsley always attributed much of his own attitude of mind to the influence Macewen had on him. In 1915, at the request of Prof. Bryce, he returned to the Department of Anatomy as temporary Senior Demonstrator, and later in 1915, his resignation having been declined, was allocated for permanent service in that Department. In 1916 he was awarded the M.D. degree with honours, and was Bellahouston Gold Medallist, for his thesis on joints and their mechanisms. The early interest in joint structure and function was to persist throughout his life. In 1918 came appointment as Lecturer in Anatomy and Embryology in succession to J. F. Gemmill. It was during the latter part of his career in Glasgow that Walmsley commenced to write his *Practical Anatomy* and, indeed, the first volume was published from there.

In 1919, and at the early age of 30, Walmsley was appointed to the Chair of Anatomy in The Queen's University of Belfast, as successor to Prof. Johnson Symington. Owing to Prof. Symington's terminal illness and the difficulties of the later war years, there had been an 'inter-regnum', and much work was required to re-establish the considerable traditions of Belfast anatomy. Working quietly and steadily, however, Prof. Walmsley soon built up an excellent teaching department. In his teaching there was always an emphasis on principles and an attempt to lessen the students' load of topographical detail. Associated with this attempt was the early publication of the second and third volumes of the *Practical Anatomy*. A post-2nd M.B. science course in anatomy, in which he insisted on a nice balance between embryology, anthropology and neurology, was also developed. This B.Sc. in Human Morphology and Embryology was to have a marked effect on the future careers of a number of his students.

During this time a second edition of the *Practical Anatomy* was published, the first volume appearing in 1934. That Walmsley was early aware of the importance of modern trends in the teaching of anatomy to undergraduates was shown by the inclusion in this new edition of sections on living anatomy and X-ray anatomy designed to synthetize for the student information gained by dissection of each region of the body.

In his elementary teaching, which he considered a most important part of his duties, Walmsley was peerless. He combined in a most striking manner, simplicity

THOMAS WALMSLEY

of presentation with impressiveness in delivery. The lectures had, indeed, an almost ritualistic, if not even a devotional character. The large classes were controlled with complete effortlessness. Even the 'toughs', the out-and-out Philistines, were completely subjugated. Yet there was never threat, mandate or persuasion—only a compelling voice and a subdued attention. Mockers did not exist either during or after his lectures. The effect must reverberate in the memories of all his former students.

In his advanced teaching Walmsley was, again, most effective. He maintained the attitude that in matters of details it was the duty of any interested persons to 'get up' the facts for themselves. The duty of the teacher of those desirous of entering the 'arcana of the higher Anatomy' was, in his opinion, to keep the facts within the bounds of reason. Each point was subjected to logical analysis against the background of Scottish metaphysics and cold common sense. He abhorred the loose statement, the definition not adhered to; and he disliked over-enthusiasm. The result was an inculcation of a critical state of mind that could even be chilling to the young and ardent, but it was an excellent training in morphological analysis. The spirit of criticism extended to the presentation of facts; indeed, in this regard, Walmsley's attention to words, and the meanings of words, seemed to the neophyte almost pedantic. It is only in retrospect, and with the awareness of what tautology and semantics have come to signify in modern knowledge, that former students of his can fairly assess his stature in the matter, and manner, of presentation of morphological investigations. Along with his critical approach he had two hobby horses—the interrelated problems of adaptation and the acquirement of characters by organisms—which he rode with discretion. Here he tended to heterodoxy, Samuel Butler being the arbiter and D'Arcy Thompson the censor.

Walmsley's research interests were wide and ranged from morphology, through topography and clinical anatomy, to anthropology and ethnology. During his tenure of the Belfast Chair, nearly one hundred contributions appeared from the Anatomy Department there. Among the contributions some twenty-five appeared under his name, including the volume on 'The Heart' in the 11th edition of *Quain's Anatomy*. Many of the remaining contributions, however, could have borne his name as the work was suggested, nurtured and edited by him. His influence on the members of his staff was considerable. Of all the reasons he had for pride in his department and its work, that which alone he ever revealed—and then only as he knew death to be approaching—was success in the subsequent careers of his advanced students and demonstrators. Of these, six were elected to professorial rank in anatomy; three hold senior lectureships and a large number are at consultant level in surgery.

It is not an easy task to write of Walmsley as an individual. His natural diffidence had combined with a positive distaste for ostentation to give a certain impression of impassiveness. Further he reacted markedly against those inclined to wear their hearts on their sleeves. There were two very distinct sides to his life. He was known to most as a scientist who attracted to himself staff and students by virtue of his standing as a biologist and anatomist. His personality gave evidence of a strong sense of devotion to a mode of life and work. This was very evident to all with whom he came in contact and especially did it make a profound impression on young and eager minds. To those who sought the way of knowledge he pointed out the road and assisted them on the journey. Their further advance was a matter of keen personal interest to him, but not many knew at the time to what degree that interest extended. Those who were able to cross the gulf which separated his public from his private life know how human he was and how eagerly he seized the simple pleasures of living. His family gave him an all-embracing emotional satisfaction for he believed in the continuity of life as an ultimate element in the cosmic process.

To his wife, son and daughter, and to his brother, Prof. Robert Walmsley of St Andrew's University, we offer our sincere sympathy.

J.D.B.
J.S.B.

ALBERT PEACOCK, M.Sc., M.B., M.R.C.S.

The death of Dr Albert Peacock at the age of 46 is a sad loss to anatomical science in this country, and to the London Hospital Medical College, where he was senior lecturer. He received his medical education at the London Hospital and qualified M.R.C.S., L.R.C.P. in 1927. For 12 years Dr Peacock was in general practice in West Ham, but during this period he still retained his interest in academic anatomy. In 1940 he sought and obtained a demonstratorship in the Anatomy Department of the London Hospital Medical College under Prof. J. D. Boyd. He was a member of this department throughout the greater part of his academic career, except for a year spent at St Bartholomew's Hospital Medical College and another year as a Commonwealth Fellow in Australia. He returned to the London Hospital as a lecturer in 1949. He obtained the M.B., B.S. degrees (London) in 1940 and the M.Sc. degree in 1948.

Dr Peacock possessed a remarkable knowledge of many branches of anatomy, in addition to human topography which he taught so well. His special interests lay in the normal structure, pathology, development and evolution of the axial skeleton. His published work deals mainly with the human vertebral column; his recent paper 'On the development of the intervertebral discs' published in the *Journal of Anatomy*, is an important contribution. Another paper on the subject had been almost completed at the time of his death. He had plans for a survey of the morphology of the notochord and intervertebral discs in chordates. His approach to his subject was both enthusiastic and critical; his wide knowledge of the literature was facilitated by a fine command of languages, expecially German.

In addition to his scientific studies, Dr Peacock had many other interests, the chief of which was music. He was a talented 'cellist and pianist, and many of his friends must have enjoyed the evening performances which he used to give in his home.

An essential feature of Dr Peacock's character was his friendliness and kindness to all whom he encountered, and his capacity for making friends with different types of people in diverse walks of life. He will be remembered by those who knew him as a congenial companion and a most entertaining conversationalist, with an inexhaustible fund of fascinating anecdotes, often relating to forensic aspects of his medical practice in London. He was, moreover, a unique personality, both forceful and attractively diffident, with an endearing touch of personal eccentricity; others have written of his familiar mannerisms, the enormous pair of forceps he carried in the dissecting room, the ready and powerful handshake. Dr Peacock's many friends will wish to convey to his wife their feeling of loss, and their deepest sympathy.

A.d'A.B.

REVIEWS

Cunningham's Text-Book of Anatomy, 9th edition. Editor: J. C. BRASH. (Pp. 1604, with 1252 text-figures and 88 plates; £4. 10*s*.) Oxford University Press. 1951.

The appearance of new editions of anatomical text-books has become such a regular and frequent event that they often pass unnoticed in the journals devoted to the subject. This happened, so far as the *Journal of Anatomy* is concerned, with the 8th edition of *Cunningham's Text-Book*, which appeared during the war under the joint editorship of Prof. J. C. Brash and Dr E. B. Jamieson. The present is therefore an appropriate moment to welcome the appearance of the 9th edition, particularly as the passage of time has led to the retirement of the co-editor and the loss of several previous contributors.

The 9th edition is published under the sole editorship of Prof. Brash; Dr R. G. Inkster has taken over the section on osteology, Prof. R. Walmsley, arthrology, and Sir James Learmonth, surface and surgical anatomy. Other contributors remain as before, but one must note with regret that the obituaries of two among them, Prof. Appleton and Prof. West, have appeared recently in this *Journal*.

Despite these changes, the general arrangement and content retains the form which is familiar to all British anatomists, and needs no description here. One need not look far, however, to find numerous alterations and additions, so that, although still *Cunningham's Text-Book*, this edition can fairly be described as a modern work, which, within inevitable limits, has kept pace with recent developments. A well-illustrated account of the early development and implantation of the human embryo, based principally on the work of Hertig and Rock, which has greatly improved the embryological section, and Prof. Grant's account of the bronchial tree and the broncho-pulmonary segments, revised along the lines of modern work, are notable changes; but all sections have seen extensive revision and by no means always in matters of detail. The number of illustrations has been increased, and many redrawn or replaced.

These, however, are the sort of improvements which one has come to expect in new editions, and to quote the Preface, 'the most important innovation...is the provision of a List of References at the end of each Section'. The time is long passed when any single volume can deal comprehensively with all aspects of human descriptive anatomy and embryology, and guidance in the maze of original sources is the only solution to this difficulty. The references given are naturally for the most part to work done in the last ten or twenty years, but older papers and monographs are adequately represented, and each list is a well-selected bibliography of its subject. While one may doubt whether many medical students will find the time to make as much as one could wish of these lists, they will be of great value to those who teach them, and will extend the scope of the book to students who are working for the B.Sc. degree and for post-graduate degrees in anatomy.

The Editor and Contributors are to be congratulated that so much has been accomplished without enlarging the book by more than 46 pages. It is clear that the 9th edition of *Cunningham's Text-Book* will retain the popularity earned by previous editions, and that its value as a standard work of reference has been considerably increased. F. GOLDBY

Die gesunde und kranke Wirbelsäule im Röntgenbild. By G. SCHMORL and H. JUNGHANNS, 2nd edition, XII. (Pp. 281, 299 figures; bound, D.M. 69.) Stuttgart: G. Thieme Verlag. 1951.

The contributions of the late Prof. Schmorl to our knowledge of the vertebral column were based on the meticulous examination of an enormous amount of post-mortem material. *Everything* was studied in *every* autopsy. The results, therefore, are a fundamental contribution as much to normal anatomy as to pathology. In the first edition, which appeared in 1932, of the volume under review Schmorl had the assistance of Dr Junghanns who was a practising radiologist trained in the pathological techniques of the Schmorl school. The resulting collaboration produced a volume that has had a considerable influence on generally accepted views concerning the structure and function of the vertebral column and even more on the diagnosis and treatment of vertebral disease. Indeed the investigation stimulated by the first edition of the Schmorl and Junghanns book made it inevitable that a second edition, in which the work thus stimulated could be integrated with the new classic interpretations of the first edition, would eventually be necessary.

For the anatomist the most interesting sections of the new edition will be those on the growth and normal structure of the vertebral column, and its elements; and on defective development of the column. These two sections have been brought up to date, particularly so far as the German and Swiss literature is concerned, and in them anyone interested in the vertebral column will find much that is new and a refreshingly functional attitude. The illustrations, particularly the skiagrams, are excellent and the descriptions are clear and succinct. The anatomist will feel the lack of a comparative approach and, possibly, a tendency to plunge *in medias res*—for, after all, the vertebral column is only part of a *Granzheit.* But the book is a monograph and is intended for study by those already in possession of an adequate background. For such it is difficult to be other than enthusiastic in recommendation of this edition of a classic and fundamental contribution to our knowledge of the human body. The volume may not altogether fulfil the requirements of formal morphology; nevertheless it demonstrates in the clearest manner imaginable the contribution that structural study can make to the knowledge of the cause, the results, and indeed, the treatment of disease.

In the sections of the volume which treat of the specific diseases of the vertebral column and of the inter-vertebral discs, there is much to interest an anatomist. It is hardly, however, within the morphologist's purview to attempt an assessment of their virtues or shortcomings. The book closes with thirty large pages of closely printed references. The presence of a well-set-out and detailed 'contents' does not compensate for the absence of an index.

J. D. BOYD

Grundriss der Histologie und mikroskopischen Anatomie des Menschen. Biologie der mikroskopischen Grössenordnung. By H. PETERSEN. Edited by FRITZ KÖRNER. (Pp. 177; 196 figures; 8 × 5½ in. paper.) Berlin: Springer. 1950.

This little book is considerably more than a guide to, but less than a text-book of, histology. Its somewhat pretentious sub-title finds a certain amount of justification only in the more general parts of the book, which are by far the best; whilst some sections of those dealing with organs do not amount to much more than an orderly enumeration of the tissues that compose them. Here sometimes not even the type of muscle present in a particular region or organ is pointed out. In the case of the urinary bladder the presence of a submucosa, which is of considerable importance, is not even mentioned. The histological description of the endocrine glands cannot be called more then sketchy. As to blood, at least a reference to reticulocytes would have been indicated, and, with mammals, it would have been preferable to talk of platelets rather than thrombocytes.

On the other hand, some structural accounts (e.g. of bone, its development, the lung, and the nervous system) are—in spite of brevity—of remarkable accuracy and give much detailed information. This tendency towards extreme condensation of the text and at the same time towards completeness of representation has naturally resulted here and there in lack of clarity, and occasional passages are almost obscure, especially when suitable illustrations are missing as, for instance, in the case of the placenta, pituitary gland, ciliary body, and others. Downright inaccuracies, such as the statement that the sarcolemma is composed of collagen and reticular fibres, or that the mucous membrane of the uterine tube has folds which interconnect forming a network, are rare.

The many notable text-figures are all line drawings, few of which are reproduced from other books. Most of them, though simple in execution, are well designed, on the whole accurate and very effective illustrations. A useful coloured plate at the end of the book shows the blood cells (a group of platelets should have been included) and examples of the staining results of the most commonly employed methods.

It is perhaps not surprising to find even in this little 'Grundriss der Histologie' some reference to microcosm, holism and Weltbild, but it certainly is surprising to see the term 'cell' being derived from cellulose and also a wrong etymological explanation given of the term 'macrophage'.

F. JACOBY

An Atlas of General Affections of the Skeleton. By SIR THOMAS FAIRBANK. (Pp. xx + 411; 509 figures; 7 × 10 in. paper; 55*s*.) Edinburgh: E. and S. Livingstone Ltd. 1951.

In this volume nearly 200 cases of bone disorders have been collected to illustrate the pathology, the radiological appearance and, so far as it is known, the etiology of generalized skeletal disease. The text is confined to an etiological classification of some eighty syndromes followed by a short description of the salient clinical features of each. Treatment is not discussed. The cases quoted are fully described and profusely illustrated. Diagnosis often rests upon the X-ray alone and much care has clearly been taken to produce as complete a radiological survey as possible. It is disappointing to find that the reproduction of the films is not uniform; whilst the general standard is high, some are almost complete failures. With this exception the book is well produced and will remain a great example to those who, in retirement, look for fresh interests in medicine.

P. A. RING

Treatment by Manipulation. By H. JACKSON BURROWS and W. D. COLTART. 2nd edition (revised). 'The Practitioner' Handbooks. (Pp. 80; 27 figures; 12*s*. 6*d*.) London: Eyre and Spottiswoode Ltd. 1951.

This volume surveys the use of manipulation in the treatment of locomotor disorders. The selection of cases is briefly discussed, and some of the contra-indications listed. The manipulation of individual joints is fully discussed and the procedures are well illustrated. With the increasing use of manipulation in orthopaedics, it is a pity that little effort has been made to rationalize its use, but the book remains a useful guide to the practitioner who wishes to incorporate these methods in his treatment.

P. A. RING

Functional Mammalian Anatomy (with Special Reference to the Cat). By WILLIAM T. TAYLOR and RICHARD J. WEBER. (Pp. xi + 575; 9 × 5½ in. paper; 55*s*.) London: Macmillan and Co. Ltd. 1951.

The authors of this book have set out to give an account of the anatomy of the mammal and especially the cat, not only from the structural but also from the functional aspect. This approach has been used by the authors over a period of twenty years and they have found that it has been stimulating to the student. Right from the beginning of his biological training the student is encouraged to correlate form and function so that if at a later period he decides to become an anatomist or mammalian physiologist, he has a firm foundation on which to base his subsequent knowledge.

The book is divided into fourteen chapters. The first chapter defines common terms and also gives a useful introduction to histology. The next twelve chapters give adequate, yet not too detailed accounts of the different systems. The fourteenth chapter gives instructions for the dissection of the cat.

This book is attractively laid out and well illustrated, many of the illustrations being in colour. As a text-book for students beginning the study of mammalian zoology, this book can be highly recommended.

W. J. HAMILTON

BOOK RECEIVED

Illustrations of Regional Anatomy. By E. B. JAMIESON, 7th edition (Reprint). (Seven sections; pp. 268; 84*s*. the set.) Edinburgh: E. and S. Livingstone Ltd. 1947 (reprinted 1951).

OBSERVATIONS ON THE DEVELOPMENT OF THE LOWER END OF THE VAGINA IN THE SHEEP

By D. BULMER
Anatomy School, University of Cambridge

The embryology of the lower end of the vagina has been extensively studied by many workers. Varying descriptions of the parts played by the Mullerian ducts, the Wolffian ducts and the urogenital sinus in the formation of this region in different animals, and frequently in the same animal, have been given. The differing methods of development which have been described in man have been classified by Bloomfield & Frazer (1927) into three groups:

'(*a*) Descriptions which give the derivation of the vagina and hymen as purely Mullerian.

'(*b*) Those which make it mainly Mullerian, but bring in an additional element in the development of its lower part. This new factor may be derived from the Wolffian ducts or from the urogenital sinus, entailing an associated origin for the hymen.

'(*c*) Those which derive the vagina mainly or altogether from the urogenital sinus, with similar hymeneal origin.'

These three groupings may equally be used to classify the varying methods of development which have been described for the lower end of the vagina in the Mammalia.

Various descriptions of vaginal development amongst the Ungulata have been given. In a pig embryo of 125 mm., Jones (1914) described the fusion of the Wolffian ducts to form a median Wolffian canal, intervening between the lower end of the Mullerian utero-vaginal canal and the urogenital sinus. Baxter (1934), investigating the development of this region in the pig, stated that the vagina is formed in its upper part from the Mullerian ducts, and in its lower part by a median Wolffian canal. The 'hymen' is represented by a constriction in the lumen of the vagina between these two elements, and the 'hymeneal orifice' is therefore some distance cranial to the topographical opening of the vagina into the vestibule. On the other hand, Henneberg (1922) described in the pig the splitting of the urogenital sinus into dorsal and ventral portions, constituting respectively the lower end of the vagina and the urethra, by the downgrowth of a frontal septum. This view would therefore imply that the lower vaginal segment, described by Jones and Baxter as Wolffian in origin, is formed by this frontal splitting of the urogenital sinus.

Tourneaux (1888) described the fusion of the Mullerian and Wolffian ducts to form the lower part of the vagina in the horse, while Retterer (1891) found in the horse, the pig, and the ruminants, a frontal division of the upper part of the sinus into two parts. Bergschicker (1912) investigated the development of the female genital tract in the calf. The vagina was considered to enter the vestibule by a 'vagino-vestibular canal', formed from a superior recess of the urogenital sinus. His diagrams show the Wolffian ducts entering the cranial end of this recess, and in a foetus of 80 mm. neck-rump length Bergschicker pointed out that it was impossible to decide where the Wolffian ducts started and the superior recess ended. The relations of this superior recess in the calf appear therefore to be similar to those of the median Wolffian canal, described by Baxter in the pig.

In the sheep, Mijsberg (1925) described one foetus of 125 mm., but mentioned no division of the sinus into dorsal and ventral parts. He referred to a diagram given by Schwarztrauber (1903) of a sheep foetus 90 mm. in length, and pointed out that between the 90 and 125 mm. stages there was shortening of the urogenital sinus, relative to the symphysis pubis. This, he suggested, was due not to the downgrowth of a frontal septum, but to a simple relative shortening of the sinus, similar to that described by him in the human foetus.

In the following investigation, the lower end of the vagina and the urogenital sinus have been studied in a series of sheep foetuses. This series does not permit of an exhaustive description of the development of the vagina in this mammal, but it is considered that sufficient points of interest are raised to merit their recording.

MATERIAL AND METHODS

Nine sheep foetuses were studied, ranging in size from 46 to 265 mm. crown-rump length. In these foetuses the urogenital region was dissected out entire, to include the urogenital sinus down to its opening between the labia. This was successful in all but the 80 mm. specimen. The material was fixed in formol-saline, embedded in paraffin and sectioned serially, the sections being stained either with haematoxylin and eosin or haematoxylin and Orange G. The 375 mm. specimen which was dissected for the photograph shown in Pl. 2, fig. 6, was from a still-born lamb.

DESCRIPTION OF MATERIAL

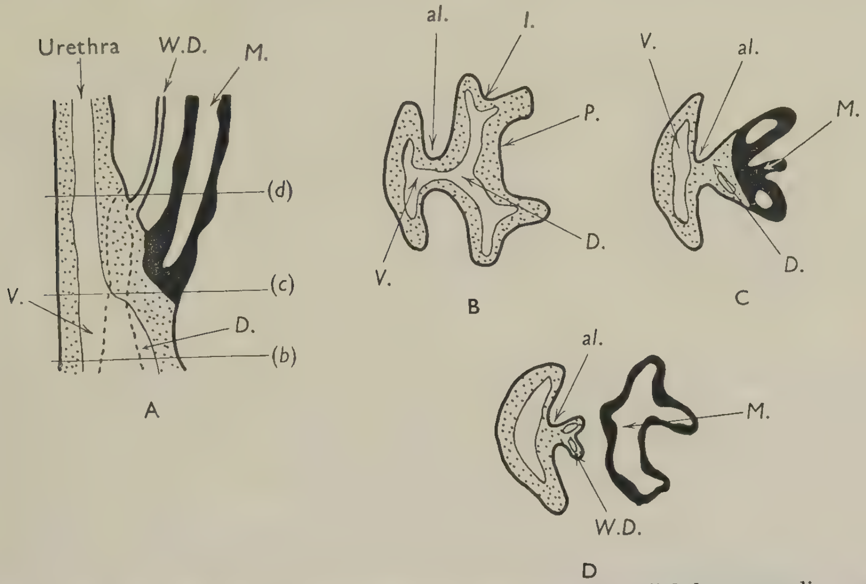
Foetus no. 1 (46 mm.)

In this specimen the Wolffian and Mullerian ducts pass medially in the genital cord. The Wolffian ducts are lined with a cubical epithelium, and are larger in diameter than the Mullerian ducts, which are lined by a more deeply staining columnar epithelium. Fusion of the Mullerian ducts occurs in the genital cord, though in places this fusion appears to be incomplete. At its lower end the Mullerian tissue consists of a solid mass, invaginating the dorsal wall of the urogenital sinus to form the Mullerian tubercle. In the lower part of the genital cord the Wolffian ducts lie lateral to the fused Mullerian ducts, and they enter the urogenital sinus by wide openings on each side of the Mullerian tubercle, their lumina being continuous with that of the sinus.

Foetus no. 2 (80 mm.)

The Mullerian ducts fuse with each other in the upper part of the genital cord. The resulting utero-vaginal canal, roughly circular in cross-section, is lined by two or three layers of columnar cells. At its lower end the utero-vaginal canal is larger, and is antero-posteriorly flattened, particularly in the median plane. At the level of its contact with the dorsal aspect of the urogenital sinus, it consists of two lateral hollow bulbs, joined by a strand of solid tissue across the mid-line (Text-fig. 1C). In this region the Mullerian cells are polygonal in shape, and their cytoplasm stains deeply with eosin. Five or six layers of these cells separate the lumen of the lateral bulb from the tissue of the urogenital sinus.

The urogenital sinus is joined above by the urethra and by the Wolffian ducts, and is in contact posteriorly with the Mullerian utero-vaginal canal. From the antero-lateral aspect of its inner wall on each side there projects a longitudinal fold, the outlines of which are shown by the interrupted lines in Text-fig. 1A. This antero-lateral fold is shown at *al.* in Text-fig. 1B–D, and together with its fellow on the other side, it incompletely separates the urogenital sinus into dorsal (*D.*) and ventral (*V.*) divisions. The ventral division is continuous above with the urethra, while at its upper extremity the dorsal division is joined by the two Wolffian ducts (Text-fig. 1D). The Wolffian ducts are now very much smaller than at the 46 mm. stage and, as can be seen in Text-fig. 1A, their entry into the sinus is at a higher level, relative to the lower end of the Mullerian vagina.



Text-fig. 1 (80 mm. specimen). A is a graphic reconstruction of a slightly paramedian sagittal section of the urogenital sinus and the lower end of the vagina. B–D are transverse sections at their respective levels shown in A. In all figures the ventral aspect is towards the left. *D.* dorsal division, and *V.* ventral division of the urogenital sinus; *W.D.* Wolffian duct; *M.* Mullerian vagina; *l.* lateral, *p.* posterior and *al.* antero-lateral folds of the sinus.

The lumen of the ventral division of the sinus is continuous with that of the urethra. In its lower part the lumen of the dorsal division is large, and communicates with that of the ventral division between the antero-lateral folds (Text-fig. 1B). Here the dorsal division of the sinus has three folds projecting from its inner wall, two lying laterally and the other posteriorly. These folds are shown at *l.* and *p.* respectively in Text-fig. 1B. At the level of contact of the utero-vaginal canal with the sinus, the lateral folds have disappeared and the posterior fold is less noticeable. A transverse section here (Text-fig. 1C) shows the dorsal division to consist of two symmetrically placed wings, each lying anterior to the lateral Mullerian bulb. The lumen is very small, and slightly above this level is completely absent.

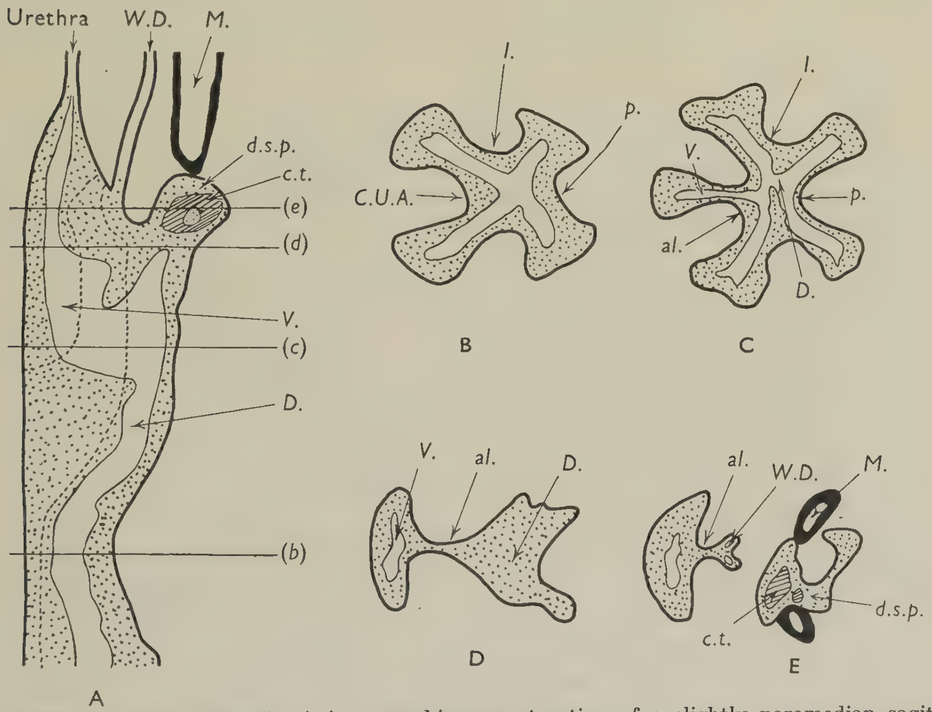
The lining of the ventral division of the sinus, and of the dorsal division where a central lumen is present, consists of a transitional epithelium five or six cells thick. The basal layer consists of cubical or low columnar cells, while the superficial layers are made up of polygonal or flattened cells with very pale-staining cytoplasm. The solid tissue of the upper part of the dorsal division is formed by a mass of very pale-staining polygonal cells, surrounded by a basal, rather more deeply stained layer of cubical cells. No clear cytological distinction can be made between the pale-staining cells of this tissue and those of the transitional epithelium described above. The solid tissue of the dorsal division is in contact posteriorly with the deeply staining tissue of the Mullerian utero-vaginal canal, and can be clearly distinguished from it. The lower end of the Wolffian ducts enter the upper extremity of the dorsal division of the sinus, and are lined by a single layer of low columnar epithelium. As these ducts are followed cranially they lose their lumina, become increasingly difficult to distinguish, and eventually disappear.

Foetus no. 3 (98 mm.)

The most noticeable feature of difference from the 80 mm. stage is the presence of an upward projection of the dorsal division of the urogenital sinus, in contact above with the lower end of the Mullerian vagina. This structure will be termed the superior projection of the dorsal division, and is shown as *d.s.p.* in Text-fig. 2A and 2E.

The lower part of the sinus (Text-fig. 2B), which was not available in the 80 mm. specimen, will now be considered. The inner wall is characterized at this level by the presence of four longitudinal folds—one anterior, one posterior, and two lateral. These folds were noted by Mijsberg in his description of a 125 mm. sheep foetus, and the anterior one was called by him the crista urogenitalis anterior (shown as *C.U.A.* in Text-fig. 2B). At a slightly higher level (Text-fig. 2C) the lateral and posterior folds are still present, while the crista urogenitalis anterior has become split into the two antero-lateral folds (*al.*). These antero-lateral folds are in a similar position to that shown in Text-fig. 1B for the 80 mm. foetus, and they bound the communication between the dorsal and ventral divisions of the sinus. In Text-fig. 2D the posterior and lateral folds are much less pronounced, while the antero-lateral folds are still present. In Text-fig. 2E the level of section passes through the superior projection of the dorsal division, which is in contact with the lower end of the Mullerian vagina. The antero-lateral folds are now narrower antero-posteriorly, and limit the connexion between the ventral division of the sinus and the cranial end of the dorsal division. Here the dorsal division is joined by the lower ends of the Wolffian ducts. At this stage, therefore, the upper part of the dorsal division may be regarded as having two parts (Text-fig. 2A). The anterior part is joined by the Wolffian ducts, while the posterior part forms the root of the superior projection. The Wolffian ducts now enter the sinus at a lower level, relative to the lower end of the Mullerian vagina, than at the 80 mm. stage.

The lumen of the lower part of the sinus, shown in Text-fig. 2B, is widely continuous above that of the dorsal division. The dorsal division communicates anteriorly with the ventral division, between the antero-lateral folds. This communication is in the shape of a vertically elongated slit, bounded below by the fusion of the antero-lateral folds to form the crista urogenitalis anterior, and above by the meeting of these folds cranial to the entry of the Wolffian ducts. The lumen of the ventral division is directly continuous above with that of the urethra. In its upper part and in its superior projection the dorsal division has no lumen, and consists of a solid mass of pale-staining polygonal cells, surrounded by a basal layer of low columnar cells. No clear distinction can be made between the cells of this solid tissue, and those of the transitional epithelium of the urethra and the lower part of the sinus.



Text-fig. 2 (98 mm. foetus). A is a graphic reconstruction of a slightly paramedian sagittal section, while B–E are transverse sections at their respective levels shown in A. *C.U.A.* crista urogenitalis anterior; *d.s.p.* superior projection of the dorsal division of the sinus; *c.t.* connective tissue. Other lettering as for Text-fig. 1.

The state of the Mullerian ducts is little different from that found at the 80 mm. stage. Followed downwards the utero-vaginal canal becomes transversely elongated, and in section shows a curve whose concavity faces dorsally. At its lower end it consists of two laterally placed hollow bulbs, joined by a strand of solid tissue across the mid-line. This solid strand lies above and posterior to the superior projection, while the two lateral bulbs, reaching down to a lower level (Text-fig. 2E), lie on each side of this projection. At the level of its contact with the Mullerian tissue, the superior projection is rather irregular in shape, appearing in section as though lobules of connective tissue have invaginated it from one side (see Text-figs. 2A and 2E, and Pl. 1, fig. 1). The tissues of the Mullerian vagina and the superior projection at their junction can be readily distinguished from each other.

The persistent lower ends of the Wolffian ducts lie anterior to the utero-vaginal canal. Traced caudally they become more readily distinguished, and leave their close relation with the utero-vaginal canal, by passing more anteriorly to enter the urogenital sinus as already described. The Wolffian duct consists of low columnar cells, and only at its lower extremity, where it actually enters the sinus, can a lumen be distinguished.

Foetuses nos. 4 (115 mm.) *and* 5 (126 mm.)

These two foetuses are at a similar developmental stage, and one description may serve for both. Micro-photographs from each are shown in Pls. 1 and 2, and Text-fig. 3 is drawn from the 126 mm. specimen. This diagram may be compared with the one shown by Mijsberg of a 125 mm. foetus.

The utero-vaginal canal is large, and below it becomes flattened antero-posteriorly. Here longitudinal ridges project from its inner wall, and between the ridges the lumen forms small pouches. At its extreme lower end no lumen is present, and the Mullerian tissue consists of a solid mass of deeply staining polygonal cells. This solid Mullerian tissue is in contact, below and anteriorly, with the superior projection of the dorsal division of the sinus (Pl. 1, fig. 2). The tissue of the superior projection is similar to that described in the previous specimen, and the line of demarcation between it and the Mullerian tissue is quite clear.

As before, the dorsal and ventral divisions of the sinus communicate with each other by a narrow longitudinal slit between the antero-lateral folds, and in the lower part of the sinus these folds fuse to form the crista urogenitalis anterior. Above, the antero-lateral folds meet each other to separate the superior projection behind from the urethra in front. The superior projection is now about 0·3 mm. in height—approximately double the measurement found at the 98 mm. stage. In transverse section its shape is roughly rectangular, with the long axis lying transversely, and the lumen of the dorsal division of the sinus does not extend into it. Under a low-power view, the tissue of the superior projection can be distinguished from that of the urethra and the rest of the sinus by the paler staining of its basal cells (Pl. 1, fig. 3). On closer examination, no more definite cytological difference can be detected.

At this stage the appearance of the remnants of the lower ends of the Wolffian ducts is of some interest. The lumen of the duct is lined as before with a cubical epithelium, but this lumen does not extend down to reach the urogenital sinus. Lying on the lateral aspect of the lower part of this Wolffian duct is a cord of solid tissue, which reaches down to a lower level than the duct itself to join the urogenital sinus. This structure, which may be called the Wolffian cord, is of similar tissue to that of the superior projection of the sinus, with very pale-staining polygonal cells surrounded by a more deeply stained layer of low columnar cells (Pl. 2, fig. 4). The Wolffian cords on each side join the anterior aspect of the superior projection. This junction occurs immediately above the level at which the antero-lateral folds meet each other to separate the superior projection behind from the urethra in front. In fact, in the 126 mm. foetus, the Wolffian cord on one side joins the upper and posterior angle of the narrow longitudinal connexion between the dorsal and ventral divisions of the sinus, which is bounded by the two antero-lateral folds. Compared with the 98 mm. stage, the junction of the Wolffian tissue with the urogenital sinus has therefore changed its position. In the 98 mm. foetus, it was possible to regard the dorsal division of the sinus as having an anterior part which received the Wolffian ducts, and a posterior part which formed the root of the superior projection. This distinction can now no longer be recognized, as the entry of the Wolffian cords has moved relatively caudally and posteriorly, to join the lower extremity of the anterior aspect of the superior projection.

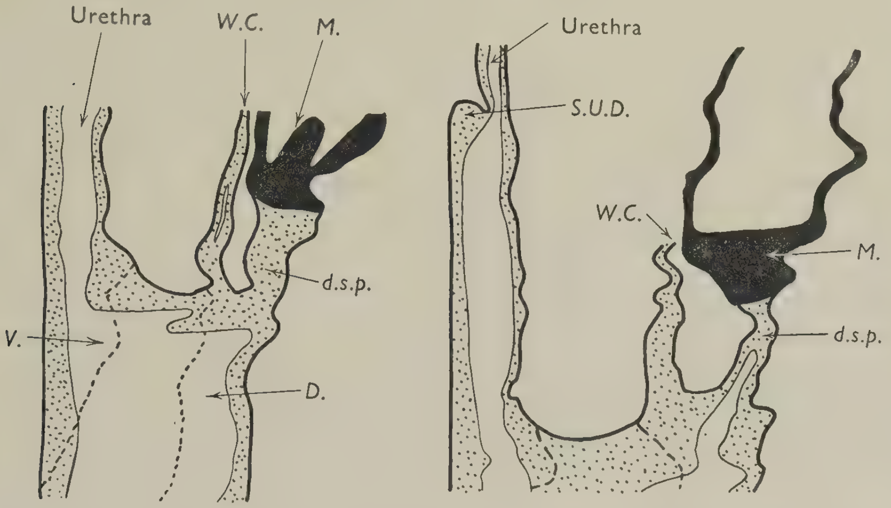


Fig. 3. Fig. 4.

Text-figs. 3 and 4. These are graphic reconstructions of slightly paramedian sagittal sections of the 126 and 152 mm. specimens respectively. *W.C.* Wolffian cord; *S.U.D.* suburethral diverticulum. Other lettering as for Text-figs. 1 and 2.

Foetus no. 6 (152 mm.)

In this specimen no important change has occurred in the relationships of the various structures. The height of the superior projection of the dorsal division of the sinus is about the same as at the 126 mm. stage. The antero-posterior narrowing, resulting in the rectangular cross-section of the superior projection, is now very marked, so that while its antero-posterior dimension is little more than half that found in the 126 mm. foetus, its transverse extent has become doubled. The lumen of the dorsal division is continued upwards into the superior projection for about half its total height (see Text-fig. 4).

In its upper part, the epithelium of the Mullerian vagina consists of a double layer of columnar cells, whose cytoplasm stains deeply with eosin. In its lower part, however, the epithelium is thicker, and shows a basal layer of deeply staining cells surmounted by five or six layers of paler cells. These pale cells, however, stain more deeply than the polygonal cells of the superior projection. This change appears to be the commencement of stratification of the vaginal epithelium, beginning from the lower end. At the extreme lower end of the Mullerian vagina no lumen is present, and the stratified epithelium is replaced by a mass of polygonal cells in contact with, but clearly demarcated from, the superior projection (Pl. 1, fig. 4).

The remains of the lower ends of the Wolffian ducts are of approximately the same length as at the 126 mm. stage. The upper extremities of these Wolffian remains are very small, and consist of radially arranged cubical cells without any central lumen. Lower down, however, a lumen, lined by cubical cells, is present, while the solid Wolffian cord lies on the lateral side of the lumen. The lumen does not reach down to the urogenital sinus, and in this specimen the Wolffian cords fuse with each other in front of the superior projection, immediately before joining the extreme dorsal and cranial angle of the narrow longitudinal opening between the antero-lateral folds. It is a point worth noting that the diameter of the Wolffian cords is increased by about one-third between the 126 and 152 mm. stages.

The subsequent development of this region, as shown by the older foetuses, may be summarized quite briefly. The stratification of the epithelium of the Mullerian vagina gradually extends upwards. By the 265 mm. stage, the epithelium 3 mm. above the level of the meeting of Mullerian and sinus epithelia still consists of a double layer of columnar cells, but below this level the epithelium has become stratified. From the 152 mm. stage onwards, there is a gradual cranial extension of the lumen of the superior projection, so that the solid tissue separating it from the lumen of the Mullerian vagina becomes less. By the 265 mm. stage, the lumen of the superior projection is separated from the clear line of distinction between Mullerian and sinus epithelia by only a very narrow layer of sinus epithelium. The solid Mullerian tissue, however, has about doubled its actual thickness since the 126 mm. stage.

The Wolffian tissue persists, but shows no further increase in size beyond the 210 mm. stage, and its appearance remains similar to that described in the 152 mm. foetus. Lying lateral to the lumen are the Wolffian cords, and these are continued down to join the superior projection just above the level of the connexion between the dorsal and ventral divisions of the sinus, or to join the upper posterior angle of this connexion itself. The lumen of the Wolffian duct does not reach the urogenital sinus.

There is considerable increase in size of all the components of the urogenital sinus. The antero-posterior narrowing of the superior projection, though still present in the 265 mm. foetus, has become much less pronounced than in the 152 mm. specimen. At the 210 mm. stage the height of the superior projection is approximately 0·35 mm., and at the 265 mm. stage 1 mm. The longitudinal folds on the inner wall of the sinus, beneath the level of the superior projection, have become more numerous and more pronounced. The folds described in the earlier foetuses can, however, still be recognized. The crista urogenitalis anterior bifurcates to form the two antero-lateral folds, which bound the vertically elongated slit through which the dorsal and ventral divisions of the sinus communicate. These folds meet each other above, between the urethra in front and the superior projection behind.

The stage of development reached at about the time of birth can be seen from

Pl. 2, fig. 6. This is a photograph of the vestibule and the lower end of the vagina in a still-born lamb 375 mm. in length. The vestibule has been dissected, and opened from the left side. A bristle has been passed through the urethral orifice, and this opening is bounded on each side by a well-marked fold. Below, this fold fuses with its fellow to form an anterior median crest in the vestibule. These two folds represent the antero-lateral folds of the earlier foetal stages, fusing below to form the crista urogenitalis anterior. The lateral fold of the vestibule may also be seen. The two antero-lateral folds meet above the urethral orifice, and the portion of the vestibule above this level represents the superior projection of the sinus. The vertical folds on the inner wall of the lower part of the vestibule become less well-marked as they are followed into the superior projection. Separating the superior projection from the smooth-walled Mullerian vagina is a marked annular constriction. Before the specimen was dissected, it was noticed that no macroscopic lumen was present at this site, though this was not submitted to microscopy. The constriction represents the position of the narrow layer of tissue mentioned in the later foetal stages, separating the lumina of superior projection and Mullerian vagina.

It may be worth mentioning that during this investigation two other structures were noticed. Text-fig. 4 shows a solid outgrowth (*S.U.D.*) from the anterior wall of the urethra. Below the level of this outgrowth, the urethra is much wider than it is above. This structure appears to be that noticed by Mijsberg at the 125 mm. stage, and which he suggested was the suburethral diverticulum.

In the 98 mm. foetus, the anterior, posterior and lateral folds of the lower part of the sinus split the lumen into two antero-lateral and two postero-lateral bays (Text-fig. 2B). From the postero-lateral bays there arises a small solid diverticulum, similar in appearance and position to the rudiment of Bartholin's gland described by Bergschicker in the calf. This structure is still present in the 173 mm. foetus, but in none of the larger specimens. It may represent a rudiment of Bartholin's gland in the sheep.

DISCUSSION

Certain of the stages in the development of the lower end of the vagina in the sheep have been described. It has been seen that between the 80 and 98 mm. stages an upward projection arises from the dorsal division of the sinus. This superior projection is in contact above with the lower end of the Mullerian vagina and appears to have two possible methods of formation.

(*a*) It may be formed by an upgrowth of the dorsal division of the sinus, at its point of contact with the lower end of the Mullerian vagina. By its upward growth it then displaces the Mullerian vagina upwards, relative to the point of entry of the Wolffian ducts into the sinus (98 mm. stage). By further growth (115 and 126 mm. stages) it carries the entry of the Wolffian ducts dorsally so that the Wolffian tissue, now solid in its lower part, joins the extreme lower limit of the anterior surface of the superior projection.

(*b*) The superior projection may be formed by the growth of a frontal septum, separating it off from the urethra in front. This septum could be produced either by downgrowth from above, or by fusion from side to side of the two antero-lateral folds. If the frontal septum grows down from above it can be supposed to be of considerable breadth, and to carry down with it the openings of the Wolffian ducts. The septum will then be complete by the 115 mm. stage, when the junction of the Wolffian tissue with the sinus has descended to the lower limit of the anterior surface of the superior projection. It is difficult to see how fusion of the antero-lateral folds from the side could produce the required septum, since a septum formed in this way would, at the 98 mm. stage, lie anterior to the opening of the Wolffian ducts. At this stage, however, the superior projection arises posterior to the entry of the Wolffian ducts into the cranial extremity of the dorsal division of the sinus (see Text-fig. 2A and 2E).

Between these alternatives it is difficult to decide. Since active growth is taking place in the sinus as a whole while the superior projection is arising, the distinction is probably not very great. There are indications, however, that active growth does occur during the formation of the superior projection. At its first appearance the projection is a mass of solid tissue, and only later does the lumen of the sinus extend into it. In addition, one might expect that if downgrowth of a frontal septum separates off the upper part of the dorsal division as the superior projection, the well-marked lateral and posterior folds in the dorsal division at the 80 mm. stage would be present in the superior projection at the 98 mm. stage. This is, however, not the case, as these folds become gradually less pronounced, and eventually disappear as the superior projection is approached.

Koff (1933) described the formation of about the lower one-fifth of the human vagina from paired sino-vaginal bulbs derived from the urogenital sinus. In their upward growth the sino-vaginal bulbs carry with them the lower ends of the Wolffian remnants, and the hymeneal fold is considered to be formed between these bulbs and the urogenital sinus. The sino-vaginal bulbs and the lower end of the Mullerian vagina fuse with each other, and cellular proliferation occludes their lumina to form a primitive vaginal plate. At first the Mullerian and sinus epithelia may be distinguished from each other, but later they fuse insensibly in the primitive vaginal plate. The development of the lower vaginal segment in the sheep may be compared with this picture described by Koff. The superior projection arises from the sinus and fuses with the lower end of the Mullerian utero-vaginal canal. There is, however, no suggestion of any bilateral bulb formation, and at first the superior projection is without any lumen. By the 115 mm. stage, the lower end of the Mullerian vagina has become occluded by the proliferation of deeply staining polygonal cells. The solid mass of tissue, arising above from the Mullerian vagina and below from the superior projection and which separates the lumina of Mullerian vagina and sinus, may be compared with the primitive vaginal plate. The sheep, however, differs from man in that at all stages it is possible to distinguish the cells of the superior projection from those of the Mullerian vagina. Canalization of this solid mass in the sheep appears, in the later foetal stages, to advance cranially by the extension of the lumen of the superior projection. Whether this continues to be the case, or whether the last part of the tissue to be canalized is the line of junction of Mullerian and sinus epithelia, it is impossible to say, since none of the stages studied has a patent vaginal canal. Upon this point depends the value of the constriction described in the 375 mm. specimen. If it represents the line of distinction between Mullerian and sinus epithelia, it may be said to correspond with the 'hymeneal constriction' described by Baxter in the pig, in the sense that it lies between the Mullerian vagina and the lower vaginal segment. If, however, it represents the most cranial part of the original solid mass of tissue occluding the vagina, it will be of entirely Mullerian origin. In neither case will it correspond with the human hymen, as described by Koff.

New interest has been shown in the development of the genital tract in the Mammalia by workers who have investigated the effects of the sex hormones on both foetal and adult animals. A wide discussion of this field would be outside the scope of this paper, so that only the main points bearing on the problem of vaginal development will be considered. The action of oestrogenic hormones on the female genital tract produces two types of epithelial response. These are classified by Zuckerman (1940) as follows:

(1) The glandular response, consisting of an orderly proliferation of columnar secretory cells. This reaction is, in the genital tract, confined to derivatives of the Mullerian ducts.

(2) The squamous response, consisting of proliferation, cornification and desquamation of stratified squamous epithelium. This reaction is produced in a wide variety of tissues, including the vagina and the urogenital sinus.

This difference of reaction between the vagina and the rest of the Mullerian derivatives led Zuckerman to question the embryological descriptions, given for many animals, of the Mullerian origin of the vagina. He pointed out that in many forms the vagina is developed from a solid cord of cells, which only later becomes canalized. Raynaud (1942*a*) described in the mouse the origin of this solid cord from the urogenital sinus, and stated that the whole of the vagina in this animal is derived from the urogenital sinus. A similar finding was also recorded by Dantchakoff (1937) in the guinea-pig. Jost (1947) describes the origin of the lower portion of the vagina of the rabbit from the urogenital sinus and indicates that in this animal the response of this lower part of the organ to oestrogens is different from that of the upper.

It was shown by Raynaud (1942*b*) in the mouse and by Burns (1939) in the opossum that the urogenital sinus epithelium gives the squamous response to oestrogenic stimulation. Zuckerman suggested, therefore, that the similarity of response of the epithelia of the vagina and the urogenital sinus indicated the origin of the vagina from the urogenital sinus. This view is also supported by experimental work on hormonal stimulation of the male genital tract. Zuckerman supposed that in those animals in which the vagina has been described as of mainly Mullerian origin, a secondary invasion of stratified epithelium from the sinus replaces the original Mullerian epithelium. In the sheep the lower vaginal segment arises from the superior projection, and a clear distinction can be found at all stages between its epithelium and that of the Mullerian vagina. From the 152 mm. stage onwards, stratification of the epithelium of the Mullerian vagina gradually extends from below upwards. The stratified cells, whose superficial layers are flattened, stain more deeply with eosin than do those of the superior projection, and they lie entirely above the line of distinction between Mullerian and sinus epithelia. There is, therefore, no histological evidence that these stratified cells in the Mullerian vagina are derived from the urogenital sinus, and no suggestion of the invasion of the Mullerian epithelium by sinus elements. It appears, therefore, that the first generation of stratified cells in the Mullerian vagina of the sheep are derived from the original columnar epithelium.

Of possible interest from the point of view of the hormonal control of the normal development of the genital tract are the solid cellular cords, found in the sheep from the 115 mm. stage onwards, associated with the lower ends of the Wolffian ducts. These structures, which have been called the Wolffian cords, are of similar tissue to that of the urogenital sinus and its superior projection. The origin of the Wolffian cords is of some interest, and it appears impossible to decide if they are formed by proliferation of the epithelium of the Wolffian ducts, or by upgrowth from the urogenital sinus.

It appears, therefore, that in the sheep the vagina is derived in its upper part from the fused Mullerian ducts, and in its lower segment from that structure which has been called the superior projection of the urogenital sinus. In all the stages described it was possible to distinguish histologically between these two vaginal elements. The final state of development of the vagina of the sheep resembles that of the pig, described by Baxter. In the latter animal, however, the lower vaginal segment is formed from the fused lower ends of the Wolffian ducts, and the relations of the Wolffian ducts to the lower vaginal segment differ in the two animals. From the material described above in the sheep, it is not possible to decide if in the early stages of development the Wolffian ducts play any part in the formation of that portion of the urogenital sinus from which the superior projection arises. It is hoped to make this problem the subject of a further contribution.

SUMMARY

1. The structure of the region of the lower end of the vagina and the urogenital sinus in a series of sheep foetuses is described.

2. During development, a superior projection arises from the dorsal division of the urogenital sinus. This superior projection is in contact above with the lower end of the Mullerian vagina, and forms the lower vaginal segment.

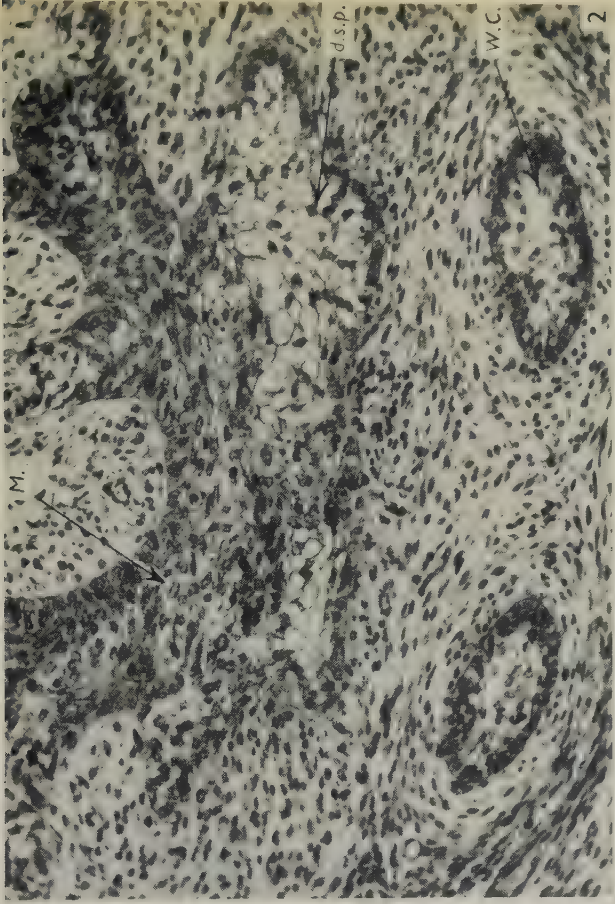
3. In a still-born lamb of 375 mm., there is no macroscopic communication between the lumina of the Mullerian vagina and the superior projection. The significance of this uncanalized tissue is discussed.

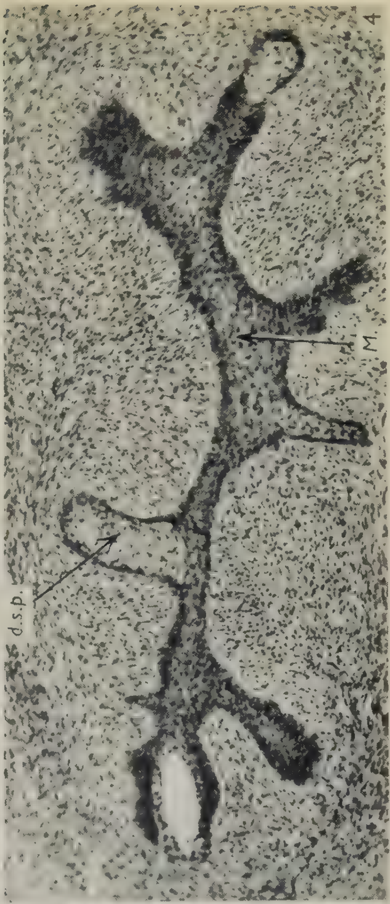
4. Stratification of the epithelium of the Mullerian vagina extends upwards from the lower end and was first seen at the 152 mm. stage. There is no indication that this stratified epithelium is derived from the urogenital sinus.

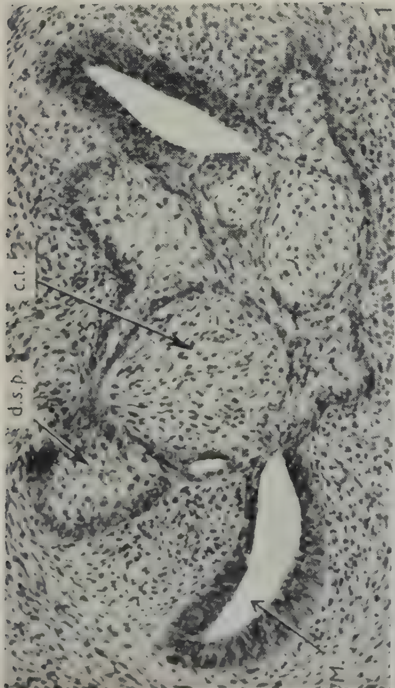
5. From the 115 mm. stage onwards the lower ends of the Wolffian ducts are associated with solid cellular cords, of similar tissue to that of the superior projection of the sinus. It is not possible to decide if these Wolffian cords are derived from the Wolffian epithelium or from the urogenital sinus.

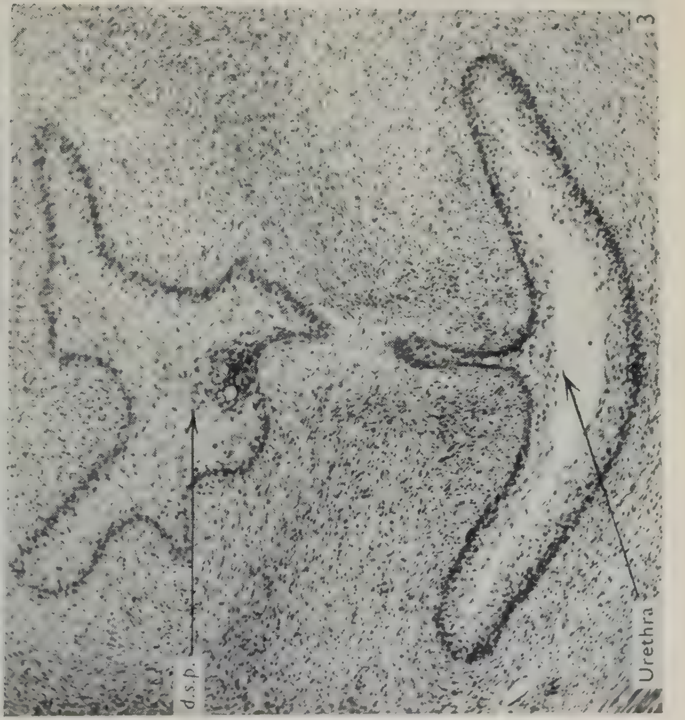
6. The presence of the suburethral diverticulum and of a structure probably representing a rudiment of Bartholin's gland are noted.

I acknowledge the kind advice of Prof. H. A. Harris and the help which has been given to me by Prof. J. D. Boyd in the preparation of this paper. My thanks are also due to Dr J. Davies for his encouragement, and to Mr J. A. F. Fozzard for the photographs on Pls. 1 and 2.

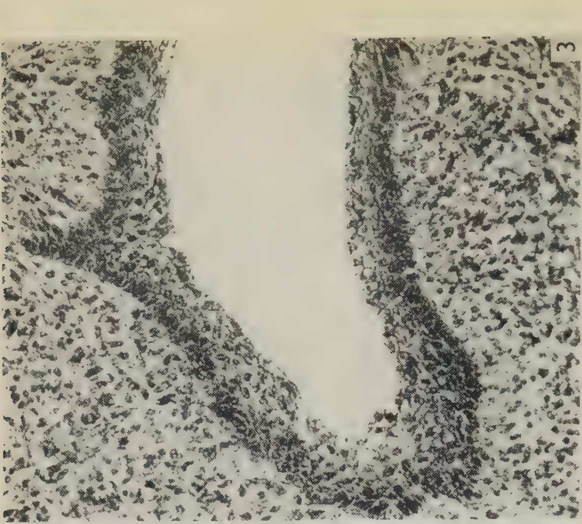








BULMER—DEVELOPMENT OF LOWER END OF VAGINA IN SHEEP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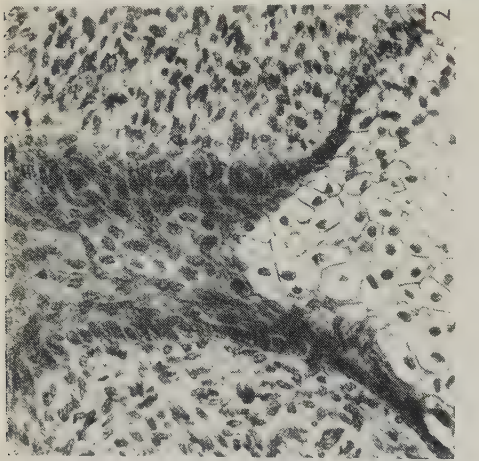
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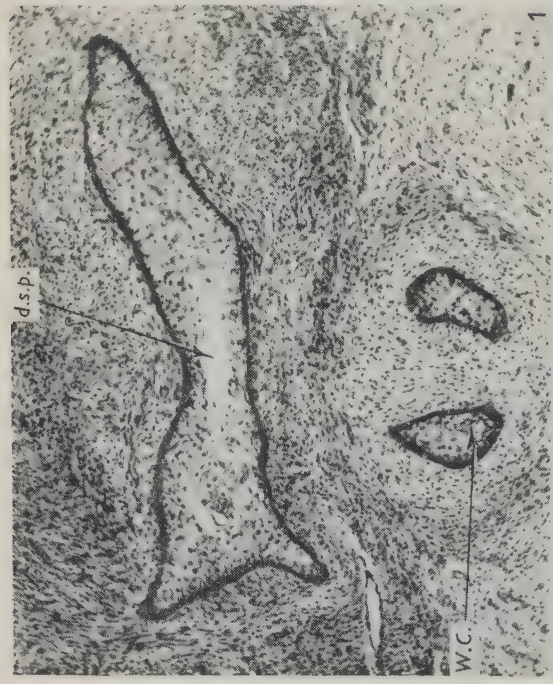
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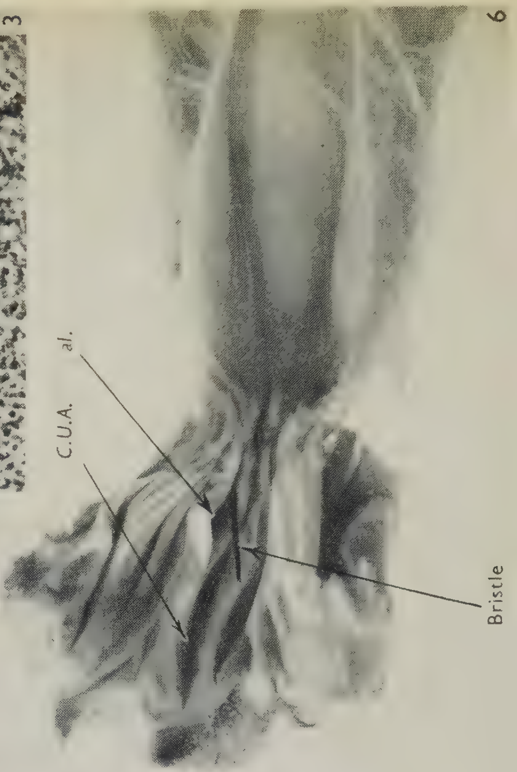
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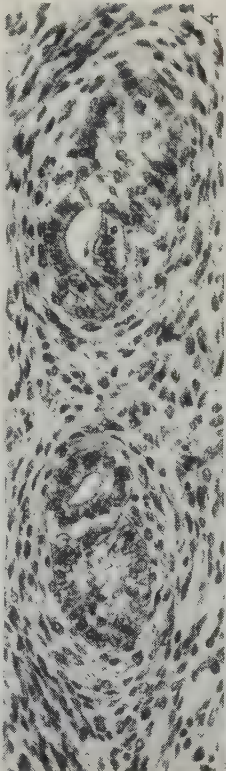
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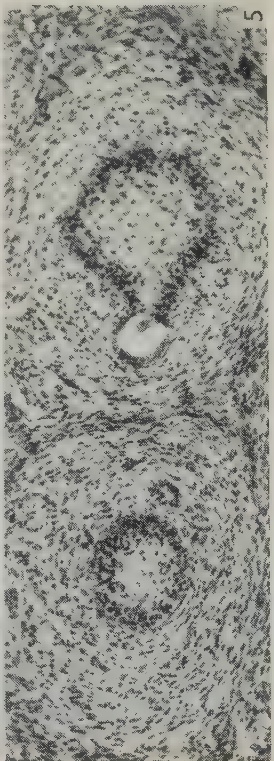
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EXPLANATION OF PLATES

Plate 1

Fig. 1. Section through the upper part of the superior projection and the lateral Mullerian bulbs in the 98 mm. foetus. (×154.)

Fig. 2. Section showing the junction of the superior projection with the solid Mullerian vagina at the 115 mm. stage. (×241.)

Fig. 3. Section through the urethra and the lower part of the superior projection in the 126 mm. foetus, showing the deeper staining of the basal cells of the urethral epithelium. (×112.)

Fig. 4. Junction of the Mullerian vagina with the superior projection in the 152 mm. foetus. (×112.)

Plate 2

Fig. 1. Superior projection and Wolffian cords at the 152 mm. stage. (×112.)

Fig. 2. Section showing the distinction between the pale-staining epithelium of the superior projection and the deeply staining Mullerian epithelium in the 210 mm. foetus. (×245.)

Fig. 3. Stratified epithelium of the Mullerian vagina in the same foetus as fig. 2. (×168.)

Fig. 4. Wolffian ducts and Wolffian cords at the 126 mm. stage. (×280.)

Fig. 5. Wolffian ducts and cords at the 210 mm. stage. (×112.)

Fig. 6. Dissection of the vestibule and vagina in a 375 mm. still-born lamb. A bristle has been passed through the urethral orifice. (×2$\frac{2}{3}$.)

ASYMMETRICAL GROWTH OF NEURO-EPITHELIA IN THE HUMAN LABYRINTH

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INTRODUCTION

It has long been known that the sensory epithelium of the vertebrate inner ear is supplied by nerve fibres of different thickness. The earliest workers studied chiefly the ampullary cristae, where they found that the periphery was supplied by thin fibres and the central ridge by thick fibres ending in calyces surrounding the bases of the hair cells. Lorente de Nó (1926), who investigated the labyrinthine nerve endings in mice using Ramón y Cajal's silver technique, was the first to describe regional differences in the innervation of the maculae. He divided the utricular macula into three zones: area *a* along the anterior and lateral margins, supplied by medium and thin fibres ending in small calyces; a central crescentic area *b* sharply demarcated from *a*, supplied by thick fibres each ending in several large calyces; and a postero-medial area *c* merging into *b* and *a*, supplied by medium fibres forming calyces and a plexus of horizontal collaterals orientated so that the fibres run transversely. The macula of the saccule in spite of its multiple innervation varies less in its internal structure. The anterior or 'dorsal lobe' of de Burlet (1924), supplied by the superior saccular nerve of Voit, contains the thickest fibres. The medium fibres of the central region break up into a rich plexus and so come into contact with a large number of hair cells. The posterior end is similar, but the fibres are thinner. The structure is not strictly comparable with that of the utricular macula, but the anterior end has certain affinities with area *b*, and the rest resembles area *c*.

Poljak (1927*a*) confirmed most of these findings in various mammals using Golgi's method. He held that the various types of ending differentiate from a common basic type, which originally is diffusely distributed in a symmetrical manner. Both Poljak (1927*b*) and Lorente de Nó (1933) have distinguished different types of innervation in the organ of Corti. The inner hair cells receive radial fibres which take a straight course and end on relatively few hair cells. The outer hair cells, on the other hand, are supplied by spiral fibres which run a long distance towards the vestibular end of the cochlea and give off collaterals which make contact with a large number of cells. There are also spiral bundles of very thin fibres under the inner hair cells.

In his earlier investigation, which included many embryonic stages, Lorente de Nó (1926) found that the cochlea becomes innervated late. As the neuro-epithelium begins to differentiate, the ganglion cells migrate centrally, and fibres of the subepithelial plexus invade the epithelium of the basal coil. The fibres to the inner hair cells precede those to the outer, and the process spreads proximally and distally along the cochlear duct. Lorente de Nó believed that in the apical region the cells at the time of invasion are highly differentiated and so ready to receive their innervation, whereas at the more rapidly growing base the fibres enter in excess and await the further multiplication and development of immature sensory cells. The vestibular nerve fibres invade the ampullary cristae before the maculae. Lorente de Nó regarded the macula of the utricle as being developmentally two separate organs: areas $a+b$ of the adult being derived with the cristae from the 'primitive embryonic macula'; while area c is formed later with the macula of the saccule. Areas $a+b$ continue to resemble the cristae in their internal structure. The structural differences which later characterize these areas are not obvious before birth. By contrast, the macula of the saccule, in spite of its double nerve supply, originates as a single organ.

de Burlet (1929) concluded, on phylogenetic rather than on embryological grounds, that the various receptor areas of the inner ear separate off from an originally single neuro-epithelial plaque in an order which is reflected by the branching pattern of the auditory nerve. Thus the primitive sensory epithelium is said to undergo a primary division into upper and lower parts. The upper then divides secondarily into the two anterior cristae and the macula of the utricle, all supplied by the upper division of the auditory nerve; while the lower part gives rise secondarily to a number of end-organs supplied by the inferior division of the auditory nerve which differ in different groups of animals, but in man consist of the macula of the saccule, the posterior crista and the organ of Corti.

In addition, the lower part of the human labyrinth has sometimes been accredited with other specialized receptors. According to Benjamins (1913), the 'macula neglecta' of lower forms occurs also in mammals (including man) at the posterior end of the utricle, as a structure which, being capped by a cupola, he preferred to call a 'crista quarta'. Lorente de Nó (1926) could find no such organ in mice, although the posterior ampullary nerve did send a few fibres to isolated cells of the utricular epithelium. Kolmer (1926) observed nerve fibres below a thickening in the wall of the ductus reuniens in a 5 months' human embryo, which he identified with a macular rudiment thought by Alexander (1901) to develop at the saccular end of the ductus and to disappear before acquiring an innervation. Recently, Palumbi (1950) reported a new sensory nervous apparatus in man, taking the form of arcades derived from apical fibres of the cochlear nerve below the undifferentiated epithelium of the blind end of the cochlear duct.

A number of authors have recorded aberrant nerve endings in the inner ear. Ramón y Cajal (1919) believed that the original entry of the vestibular fibres into the epithelium is brought about by a diffuse neurotropic action exerted by the primordial supporting cells. Under their influence exploratory fibres invade the epithelium in excess: those which do not find an attachment as a result of a specific neurotropic influence exerted by the developing hair cells are destined to be absorbed; some that have temporarily escaped this influence may, however, loop up to the surface of the epithelium. Kolmer (1926) described many such fibres in the organ of Corti of a 5 months' embryo: arching on to the surface of the membrana reticularis, along the top of the pillars of Corti and among the developing sustentacular cells of Deiters, and apparently ending in a small swelling. Tschernjachiwsky (1929–30) found similar *fibres égarées* in the macula of the utricle of a 3 months' human embryo, entering the endolymphatic space. Although the bundle from which they are derived contains both thick and thin fibres, the fibres actually piercing the epithelium to reach the utricular lumen are all thick.

In view of the absence in the literature of a comprehensive account of the stages by which the acoustic neuro-epithelium in man comes to acquire its complex definitive pattern, there seems to be a need for further study of human material. Serial sections have therefore been examined, and graphic reconstructions made, from six human embryos of different ages up to about 100 days, impregnated in bulk with silver by de Castro's technique, from Prof. J. D. Boyd's collection. Certain nervous pathways have been interpreted in accordance with a previous investigation (Shute, 1951), and a corresponding terminology has been adopted. The *superior utricular nerve* is a bundle of fibres, so named from its position before the rotation of the utricle, running to the utricular macula by way of the *common ampullary nerve* to the superior and lateral cristae. The lower trunk of the *main utricular nerve* arises from the inferior vestibular ganglion above the origin of the *main saccular nerve*. Through this trunk the *superior saccular nerve* traces its origin largely back to the inferior vestibular ganglion. The *cochleo-saccular nerve* is entirely derived from the inferior vestibular ganglion, but runs part of its course to the saccular macula in company with the *vestibulo-cochlear anastomosis*. This latter bundle is a relic of the original connexion between the vestibular and cochlear ganglia, and is composed of central processes of cochlear ganglion cells.

OBSERVATIONS

Embryo (17·5 mm.)

The otic vesicle is not yet divided into utricle and saccule. The epithelium of the original medial wall consists of several cell layers, but has differentiated into a neuro-epithelium proper only at the cristae. Below, the saccular nerves have grown towards the undifferentiated epithelium anterior to the trunk of the posterior ampullary nerve. Superiorly, the main utricular nerve forms a subepithelial plexus which extends round the front of the otic vesicle to the lateral side, where the superior utricular nerve underlies the undifferentiated epithelium adjoining the lateral crista (Pl. 1, fig. 1). All the cristae have developed a superficial layer of columnar cells at this stage, and the ampullary nerves have invaded the epithelium *en masse* so that their terminal fibres run in closely packed ranks towards the base of this primordial hair-cell layer.

Embryo (30 mm.)

Areas of neuro-epithelium have now appeared in the utricle and saccule. Between the two chambers there is a shelf on to which the utricular macula extends from the anterior wall; an area of undifferentiated epithelium intervenes between it and the lateral crista (Pl. 1, fig. 2). The superior saccular nerve has not yet invaded the epithelium above the saccular macula, which extends as a narrow strip down to the many-layered undifferentiated epithelium of the commencement of the cochlear duct (Pl. 1, fig. 4). The cochleo-saccular nerve runs direct to supply its lower end. Within the epithelium many fine fibres run transversely from the base towards the surface and ramify beneath the primordial hair cells. The cochlear nerve forms a poorly stained plexus below the two turns of the cochlear duct. No fibres penetrate the epithelium.

Embryo (43 mm.)

Below the cochlear duct, which is now commencing its third turn, the nerve plexus has thickened and shows a progressively increasing affinity for silver proximally from the apex. The epithelium of the apical and middle coils is still undifferentiated and contains no nerve fibres. In the basal coil, for some distance on either side of the point where the vestibulo-cochlear anastomosis leaves the cochlear ganglion, certain of the superficial cells have become columnar, and a few nerve fibres have pierced the basement membrane. These fibres are thin, of even calibre, and may divide at the base of the epithelium or within it, the subdivisions continuing towards the base of the differentiating hair cells. At one point an aberrant fibre, rather thicker and more darkly staining than its fellows, passes between the columnar cells to the surface of the epithelium, where it ends after a short course along the inner wall of the cochlear duct (Pl. 1, fig. 5).

The saccule has grown posteriorly and the epithelial wall of its posterior end has become relatively thin (Pl. 1, fig. 3). The neuro-epithelium of the saccular macula occupies the anterior end of the medial wall of the saccule, and is skirted posteriorly by a wide expanse of undifferentiated epithelium continuous with that of the commencement of the cochlear duct. There is therefore still no true ductus reuniens. The saccular macula now has two lobes set at an angle to one another, as shown in Pl. 1, fig. 8: the superior saccular nerve has invaded the epithelium, by this stage. The main saccular nerve enters the macula from its anterior end: the cochleo-saccular nerve loops up to it from below, but is too small to contribute significantly to its innervation.

The utricular macula has come to lie entirely at the anterior end of the lateral wall of the vertically flattened utricle. Along its upper border a strip of undifferentiated epithelium overlies the common ampullary nerve (Pl. 1, fig. 8), and another strip extends along the lower border of the macula medial to the saccule towards the developing utriculo-endolymphatic duct (Pl. 1, fig. 3). The sub-epithelial nerve bundles send many parallel fibres across the epithelium, which form small swellings or 'cones of growth' deep to the columnar cells. These swellings give off short terminal fibrils some of which run transversely as an intra-epithelial plexus, while others begin to surround the bases of the developing hair cells. Posteriorly, the macula of the utricle gives place to non-sensory epithelium which extends back to the ampulla of the posterior semicircular duct.

Embryo (48 mm.)

Calyces are beginning to form in all the vestibular areas, especially near the sites of entry of the nerve trunks. Within the epithelium some of the fibres running directly across have thickened and branch at the bases of epithelial cells into two or more enveloping arms. The saccular macula is now separated from the commencement of the cochlear duct by the newly differentiated low epithelium of the ductus reuniens. There is growing from the inner wall of the basal coil of the cochlear duct a small epithelial diverticulum which is not innervated (Pl. 1, fig. 7).

Embryo (62 mm.)

The spiral of the cochlear duct, although not yet full size, has now completed all its turns. The epithelium of the apex has reached about the same degree of differentiation as that of the basal coil at the 43 mm. stage. The apical ganglion cells still lie close to the duct, and between the two there is a rich nerve plexus. The few nerve fibres that have invaded the epithelium of the apical coil have not penetrated beyond its deeper layers. Meanwhile, the increased growth of the cochlea has resulted not only in a lengthening of the duct but also in a widening of its spiral, so that the diameter of its base is greatly increased. Through the newly formed spiral lamina the fibres of the original subepithelial plexus run out radially from the ganglion cells to the organ of Corti. Sections tangential to the cochlear ganglion show dense bundles of spiral fibres, mainly on its outer surface. They are thinner and less heavily stained than the radial fibres, and at this stage resemble in their immature appearance the fibres of the vestibulo-cochlear anastomosis.

The more proximal parts of the middle cochlear coil receive fine nerve fibres, more numerous in the inner hair cell region, running to the bases of the primordial hair coils. One *fibre égarée* enters the lumen of the cochlear duct. In the basal coil the neuro-epithelium proper begins just distal to the entry of the ductus reuniens, and becomes increasingly differentiated up to the site of entry of the vestibulo-cochlear anastomosis. The sensory cells of the organ of Corti have acquired cilia and larger nuclei than the adjacent supporting cells. Many nerve fibres divide at the level of the basement membrane, and end in cones of growth from which one or more terminal filaments ascend to the sides of the hair cells. The inner hair cell region appears to receive a denser innervation than the outer hair cells (Pl. 1, fig. 6). Most of the fibres to the outer hair cells run up to them from below, but some cross laterally from the inner hair cell bundle.

In the saccule, the two lobes of the macula have come to lie in the same plane on its medial wall against the otic capsule. The sensory epithelium is beginning to show regional differences in its innervation. The main saccular nerve bundles run back to the lower part of the macula towards its posterior tip where the tiny cochleo-saccular nerve enters. They furnish the overlying epithelium with a rich innervation consisting of thick fibres which run a fairly direct course to form primitive calyces beneath the hair cells, and also thinner plexiform collaterals. The portion of the macula superior to this densely innervated area, including the greater part of the upper lobe above the entry of the rather small superior saccular nerve, has a relatively sparse innervation from fewer and thinner fibres.

The utricular macula has completed the rotation which brings it into a horizontal plane, and now occupies the anterior end of the floor of the utricle. The border receiving the superior utricular nerve which was previously superior has become lateral, and extends posteriorly as far as the opening of the superior and lateral ampullae. In consequence the macula now overlies laterally the perilymphatic cistern and medially the saccule. The utricular nerves run from the anterior end towards the lateral margin of the macula, and the overlying epithelium is richly innervated in a manner corresponding to the lower portion of the macula of the saccule. The enormous medial extension of the utricular macula above the saccule has a sparse innervation recalling that of the upper portion of the saccular macula.

Embryo (92 mm.)

The epithelium of the cristae possesses a denser innervation than even the richly supplied regions of the maculae, and regional differences are less marked. Thick fibres branch in all directions, and end in well-formed calyces which, though commonest centrally, occur also along the sides of the cristae. At the extreme margins, and especially at each end of the crista, the neuro-epithelium contains mainly thin plexiform fibres. A few aberrant fibres run in the mesenchyme below the planum semilunatum of the superior crista, and one fine fibre actually enters the cuboidal epithelium.

The maculae are grossly asymmetrical with respect to their nerve supply (Pl. 2, fig. 15). Where the two nerves to the utricle enter the anterior upturned end of the macula, fine plexiform fibres can be seen within the neuro-epithelium, particularly in tangential sections (Pl. 2, fig. 12). Thick fibres with calyces occur chiefly in the middle region overlying the main nerve bundles. They can be found as far as the posterior tip of the macula, but are very infrequent in its medial extension. The saccular macula is similar in its internal structure and regional differences. The lower half is richly innervated (Pl. 2, fig. 14) and retains the former bilobed shape of the whole macula.

The spiral bundles of the cochlear ganglion are now deeply stained and contain many relatively thick fibres which arise from bipolar cells in the deeper levels of the ganglion, and eventually enter radial bundles. In the spiral lamina occasional fibres leave their original group to join a neighbouring bundle. Finally, some fibres enter a slender outer spiral bundle below the hair cells.

The sensory epithelium of the cochlear duct has become innervated from the extreme apex to a point just distal to the entry of the ductus reuniens. In the most highly differentiated part of the basal coil, single, rather thin nerve fibres run to the bases of the outer hair cells, and more fibres, both thick and thin, supply the inner hair cell region. They normally terminate in fine filaments embracing the bases of the hair cells. At one point, however, a *fibre égarée* of medium thickness loops to the surface of the epithelium. The appearances in the middle coil resemble those described for the basal coil of the previous embryo. In the apical coil, where the sensory epithelium is at a much more primitive stage, the innervation appears richer. The thin fibres to the developing outer hair cells form a loose plexus in the depths of the epithelium, while the inner hair cells are surrounded by a cluster of thin and thick fibres whose cones of growth have advanced to the cell bases, or, in one instance, beyond them into the lumen (Pl. 2, fig. 10).

The hair cells of the extreme apex or lagenar region which are in series with the inner hair cells of the apical coil possess an extremely dense innervation extending as far as the blind end of the cochlear duct—shown in longitudinal section in Pl. 2, fig. 11. The subepithelial cochlear bundles contain thin and thick fibres which bend up to run in close array across the neuro-epithelium. Many fibres divide at the level of the basement membrane. The cones of growth at the base of the hair cell layer each give off two or three fine terminal filaments which surround the hair cell usually to about half its height, but sometimes reaching the surface. There is a marginal zone of thin fibres, plexiform and few in number, which are in series with the nerves of the outer hair cells of the apical coil. The innervation of this region contrasts strongly with that of the other end of the cochlear duct. At its proximal end the organ of Corti tails off into a ridge of undifferentiated epithelium which sinks into the cuboidal wall of the ductus reuniens. A few cochlear nerve fibres reach its base, but terminate without penetrating the epithelium (Pl. 2, fig. 13).

No innervated structures have been found corresponding to a macula neglecta, a macula of the ductus reuniens, nor a sensory apparatus at the apex of the cochlea as described by Palumbi (1950). The utricle receives no innervation from the posterior ampullary nerve, and the apical fibres from the cochlear ganglion all supply neuro-epithelium. The extent of the utricular and saccular maculae at three embryonic stages is indicated by the scale diagrams of Text-fig. 1.

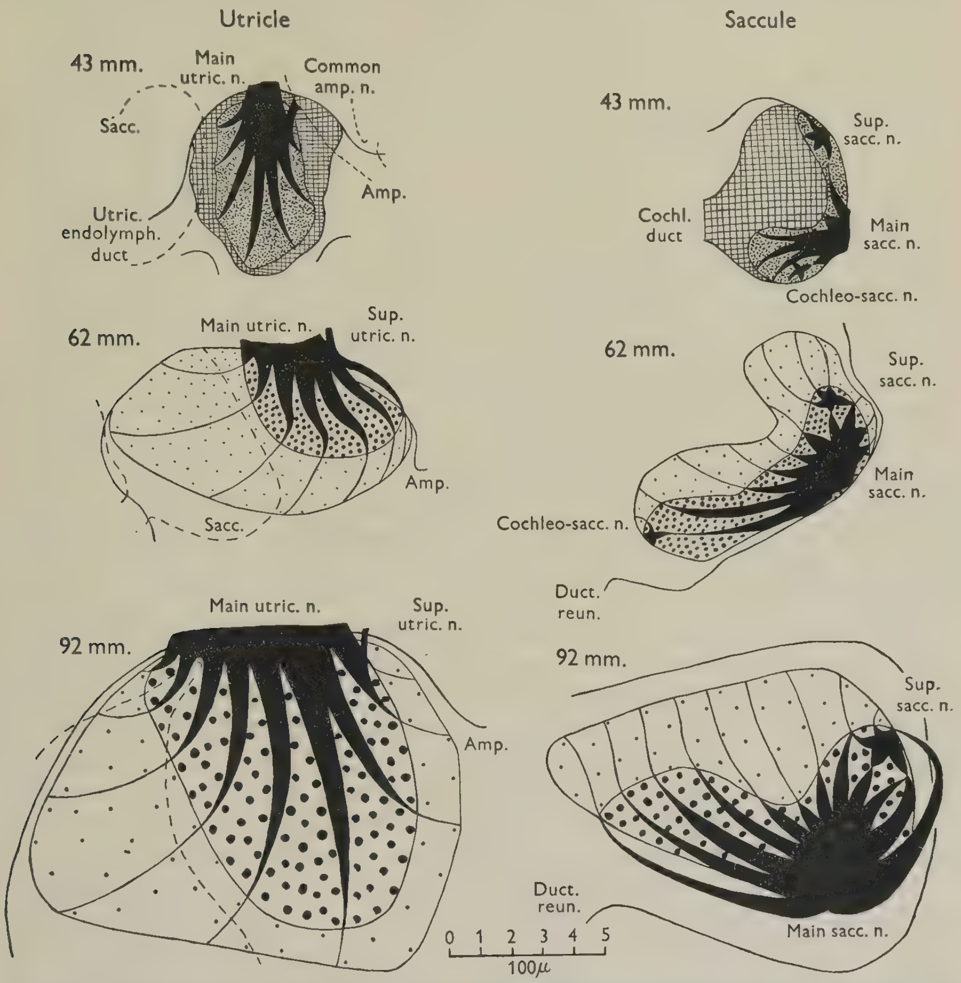
COMMENTS AND CONCLUSIONS

The distribution of the auditory nerve, particularly in its smaller branches, provides a guide to the changes undergone by the various sensory areas of the inner ear during growth. The point of entry of the cochleo-saccular nerve into the macula, near the posterior end of its inferior margin, marks the lower end of the original saccular neuro-epithelium, which extended as a strip down to the mouth of the cochlear diverticulum. Similarly, the superior saccular and superior utricular nerves mark the original upper ends of their maculae. The main direction of growth of the vestibular part of the labyrinth is backwards, away from its main innervation, and behind the neuro-epithelium there is an increasing spread of epithelium non-sensory in type. The growth of the utricle is complicated by a rotation which causes the macula to lose the support of the wall of the otic capsule, bringing it to lie above the saccule and the perilymphatic cistern. It swings about an antero-posterior axis, apparently as a combined result of the enlargement of the ampullae and the final separation of the utricle from the saccule by the formation of the utriculo-endolymphatic duct.

The basal coil of the cochlear duct elongates proximally from the point where the vestibulo-cochlear anastomosis joins it, to conform with the backward growth of the saccule. This carries the cochlear neuro-epithelium away from the most proximal cells of the cochlear ganglion, which also migrate backwards from the region of the vestibulo-cochlear anastomosis, but to a lesser extent. The epithelial diverticula which may arise anomalously from the cochlear duct just distal to the ductus reuniens are not innervated. They grow, not from neuro-epithelial territory, but from the inner wall of the cochlear duct, like spokes of a wheel; perhaps representing the failure of a group of cells to share the retarded growth of the inner side of the spiral.

The sites of future neuro-epithelium are first indicated by subepithelial nerve plexuses. Alexander's arguments for regarding a portion of the wall of the embryonic ductus reuniens as a macular *anlage*, although the cells acquire neither cilia nor innervation, are not convincing. The fact that this structure disappears entirely at a later stage when the ductus reuniens becomes narrowed suggests that it is a zone of undifferentiated non-sensory epithelium. It is therefore comparable to the thickened wall of the commencement of the cochlear diverticulum which persists in early human embryos.

The receptor areas acquire their innervation in the following order: cristae, utricular macula, saccular macula, organ of Corti. The last-named is innervated progressively in a proximal and distal direction from the vestibulo-cochlear anastomosis. This marks the point where cells of the cochlear ganglion originally began to migrate outwards and become reduced in size; it is therefore the region where the cochlear epithelium has stood longest in relation to mature ganglion cells. In all



Text-fig. 1. Scale diagrams of the maculae of the right utricle and saccule at the 43, 62 and 92 mm. stage. The utricular and saccular nerve bundles are shown black. In the 43 mm. drawings, the extent of the maculae is given by the dotted area, and the cross-hatching represents undifferentiated epithelium. In the 62 mm. and 92 mm. drawings the coarse dots represent the richly innervated portion and the fine dots the sparsely innervated portion of the macula. The positions of the saccule and the trunk of the common ampullary nerve relative to the utricular macula are indicated by broken lines.

parts of the labyrinth the terminal nerve fibres invade the epithelium shortly after the appearance of tall columnar cells at its surface. This differentiation is greatly delayed in the cochlea; until it occurs the ganglion cells remain close to the epithelium, and beneath it their peripheral processes form a dense nervous plexus—the 'expectant plexus' of Ramón y Cajal (1919). Lorente de Nó's contention, that the apical part of the cochlear epithelium becomes highly differentiated without an intrinsic innervation, is not borne out in these human embryos. Even at the apex nervous invasion follows shortly after the early signs of epithelial differentiation.

The actual process by which the nerve fibres gain connexion with the hair cells is similar in all the vestibular areas. It is only slightly modified in the cochlea, where development is delayed; nevertheless the initial innervation of the cochlear apex or lagenar region is very like that of a macula. At the first invasion a great number of fine fibres run closely packed in parallel ranks across the epithelium. Terminal fibrils emerge from the cones of growth under the hair cell layer, some of which grow up round the sides of individual hair cells, while others form a plexus beneath them. With further growth of the neuro-epithelium more fibres grow in, and the original intra-epithelial fibres become spaced out and give off branches. Some of the fibres in the nerve bundles increase in thickness, and continue to run a more or less direct course across the epithelium; they are the 'colossal fibres' of Ramón y Cajal (1908). They may divide below the basement membrane or throw off thinner, more obliquely running branches beyond it: it is a general rule that the thicker the fibre the shorter and straighter is its intra-epithelial course. Their terminal fibrils close up around the bases of the hair cells and eventually coalesce to form mature calyces.

Some 'exploratory fibres' may go astray, running up between the hair cells to the surface of the epithelium and entering the endolymphatic space. Such fibres are commonly robust, as though the product of exuberant growth. They indicate that contact with the base of a sensory cell is not a necessary stimulus for the thickening of a nerve fibre. In this series of embryos *fibres égarées* are not infrequent, but are limited to the inner hair cell area of the cochlear duct. A possible explanation is that a relatively rich complement of nerve fibres is supplying a narrow strip of neuro-epithelium only one cell wide.

The vestibular sensory areas are first discernible as uniformly innervated, discrete patches of neuro-epithelium. By the time that the proximal part of the cochlear epithelium has become innervated, the saccular macula, with the termination of the cochlear-saccular nerve, has withdrawn from it. Also, although the undifferentiated epithelium, which originally connects the utricular macula with the macula of the saccule on one hand and the lateral crista on the other, becomes invaded by the superior saccular and the superior utricular nerves respectively, the bridge of neuro-epithelium is not complete. There is therefore no 'primitive macula' in the sense of a single innervated sheet of sensory epithelium which proceeds to break up into definitive end-organs, as envisaged by de Burlet (1929).

Meanwhile, at the posterior end of the labyrinth the epithelium becomes flattened to form its definitive non-sensory wall. For a period, however, it remains separated from the maculae by an intermediate area which is still undifferentiated and has no innervation. Its extent, as is shown by the crossed-hatched areas of the text-figure, is greater in the saccule than in the utricle. Should this relic of the original thickened medial wall of the otic vesicle be regarded as future neuro-epithelium? If so, delay in neuro-epithelial differentiation would not be an exclusive feature of the cochlea, and the saccular macula in this respect would be transitional between the utricular macula and the organ of Corti. But since there is no preceding subepithelial nerve plexus, it seems unlikely that these areas are destined to receive an innervation; rather they represent a delay in the differentiation of the non-sensory wall. The epithelial cells become flattened and spaced out first at the posterior end of the labyrinth, and only later does the process reach the margins of the maculae. In the cochlea there is a comparable delay in the development of the epithelium of the internal spiral sulcus.

Eventually all the neuro-epithelia begin to show regional differences in the density of their innervation. This is most prominent in the maculae where the expanse is largest. There is a proximal region to which most of the nerve fibres run and which contains the greater number of mature endings; and a distal region where subepithelial bundles are scanty and the nerve endings within the epithelium are more scattered and tortuous. The type of nerve supply to the distal regions is not, developmentally speaking, primitive, since the neuro-epithelium initially receives a dense innervation. In the maculae, the asymmetrical arrangement recalls that of the areas mapped out by Lorente de Nó (1926) in mice on the basis of fibre thickness and type of ending. Thus, there are affinities between the sparsely innervated region of the human utricular macula and Lorente de Nó's area *c*. Both are situated postero-medially. Lorente de Nó believed his area *c* to be supplied by a separate part of the utricular nerve and to resemble in structure and origin the macula of the saccule. The postero-medial segment of the human utricular macula may come to receive a small supply from fibres of the lower trunk of the utricular nerve derived from the inferior vestibular ganglion. Lorente de Nó, however, interpreted area *c* as a separate late formation, whereas the appearances in man suggest that the sparsely innervated region of each macula arises as a result of a secondary unidirectional spread of previously formed neuro-epithelium.

Two stages can be distinguished in the development of the maculae. The primary innervation occurs while the sensory epithelium is confined to the slowly growing anterior end of the otic vesicle. The first part of the saccular macula to appear is that innervated by the main saccular nerve and is originally continuous with the undifferentiated epithelium of the cochlear duct. With the posterior growth of the labyrinth the mouth of the cochlear duct moves away from the lower end of the macula, which nevertheless continues to grow postero-inferiorly in the direction of separation. During this first stage of development the innervation keeps pace, so that the main saccular nerve bundles running back beneath the epithelium share the downward inclination of its growth. With the advance of the macula towards the more rapidly growing posterior part of the saccule the nerve bundles supplying the postero-superior segment are drawn out in the line of growth and the intra-epithelial endings become widely spaced.

The end result in the utricular macula is similar, but may be brought about differently. The part of the macula originally directed towards the lateral crista is that innervated by the small superior utricular nerve, so that only the upper margin (lateral after rotation) tends to grow towards the migrating crista. The primary growth of the macula as a whole, and of the bundles of the main utricular nerve supplying it, is directed posteriorly. The rotation of the utricle, however, which accompanies the formation of the utriculo-endolymphatic duct, leads to an enlargement medially. The medula grows away from its innervation, giving rise to a sparsely innervated postero-medial segment.

Regional differences in the density of the innervation in other parts of the labyrinth can also be correlated with the rate of epithelial growth. The neuro-epithelium of the ampullary cristae undergoes less expansion than that of the maculae, and consequently it has a considerably denser innervation, particularly in the central parts, which are situated on the relatively slowly growing anterior walls, compared with the ends of the cristae which grow backwards with the enlarging ampullae.

In the cochlea, where the neuro-epithelium bends into a spiral, the outer hair cell region must grow more rapidly than the inner and the base more rapidly than the apex. In each instance the nerve supply to the faster growing region appears relatively attenuated. While the apex of the cochlear duct is still at the stage of primary innervation, its rapidly lengthening vestibular end has outstripped its innervation. This differential growth may also account for the turn taken by the outer spiral bundles. On the other hand, the intra-ganglionic spiral bundles probably result from the great elongation that the ganglion undergoes in the course of development.

SUMMARY

1. An account has been given of the development of the end-organs of the human inner ear up to about the fourth foetal month.

2. In all parts of the labyrinth the initial invasion of the epithelium by nerve fibres is followed by growth changes which affect the intrinsic innervation pattern.

3. Scale diagrams have been constructed of the utricular and saccular maculae at three stages of development to show their asymmetrical mode of growth.

4. No evidence has been found of a macula neglecta or of a macula of the ductus reuniens occurring in man. It is suggested that no structure should be regarded as neuro-epithelial in the absence of a nerve supply.

5. Aberrant nerve endings entering the endolymphatic space have been observed only in the cochlea.

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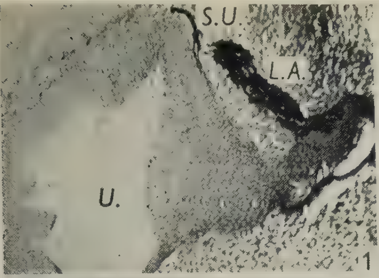

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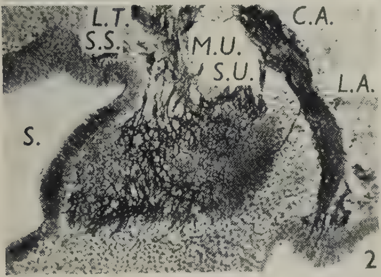

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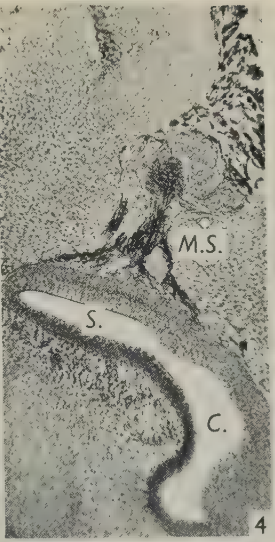

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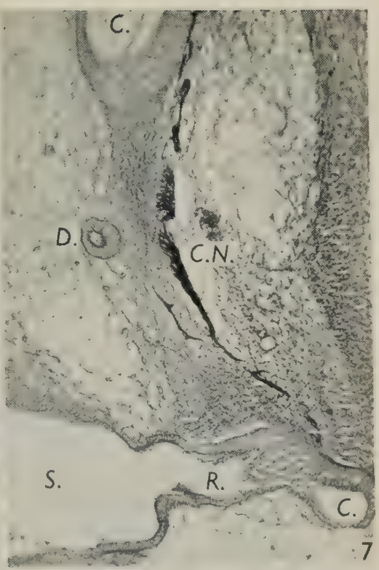

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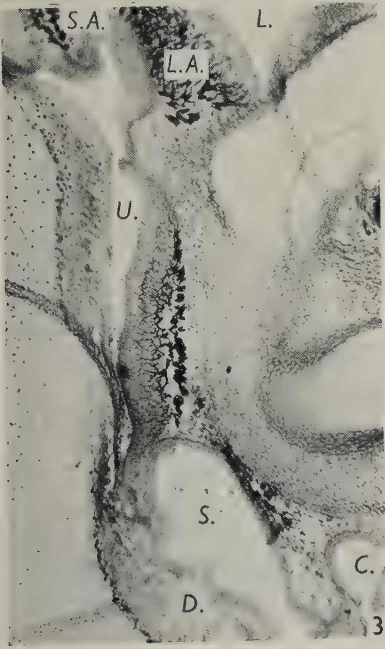

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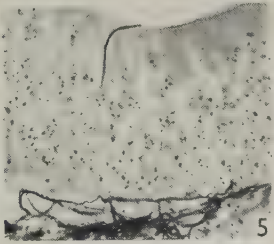

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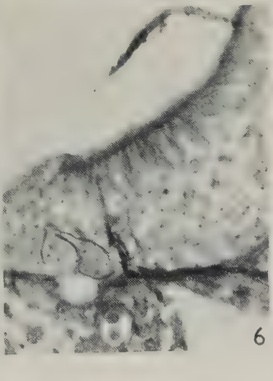

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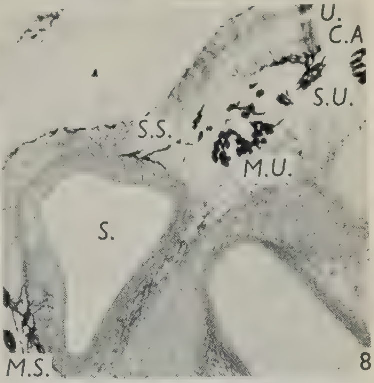

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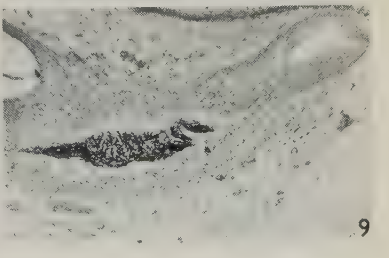

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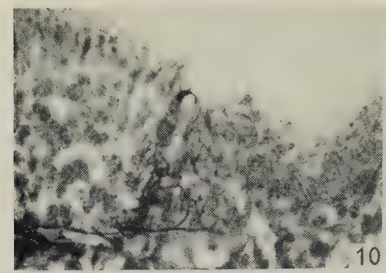
SHUTE—Growth of neuro-epithelia in the human labyrinth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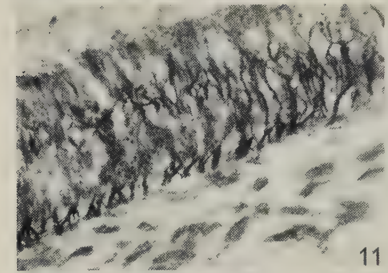
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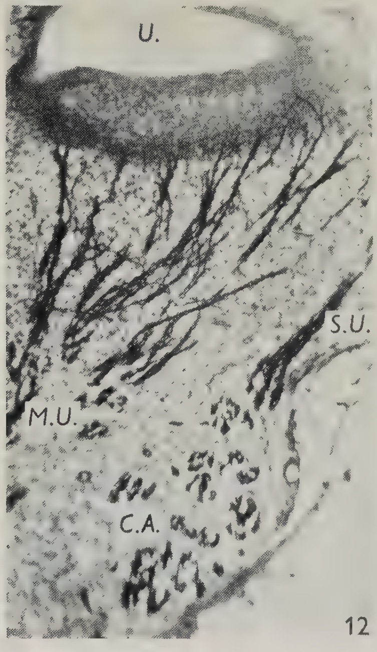

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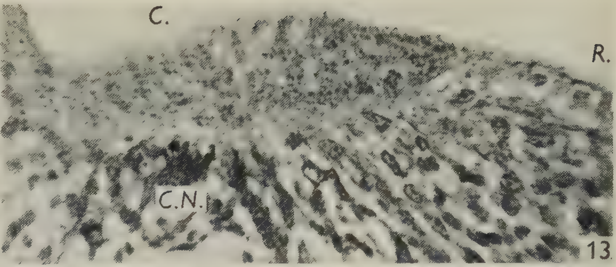

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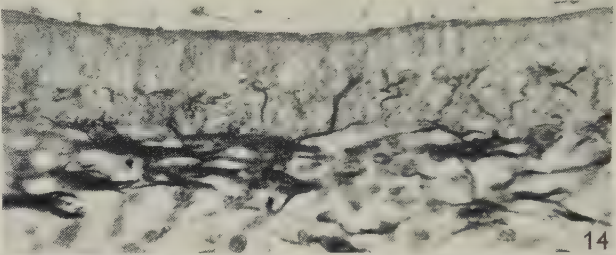
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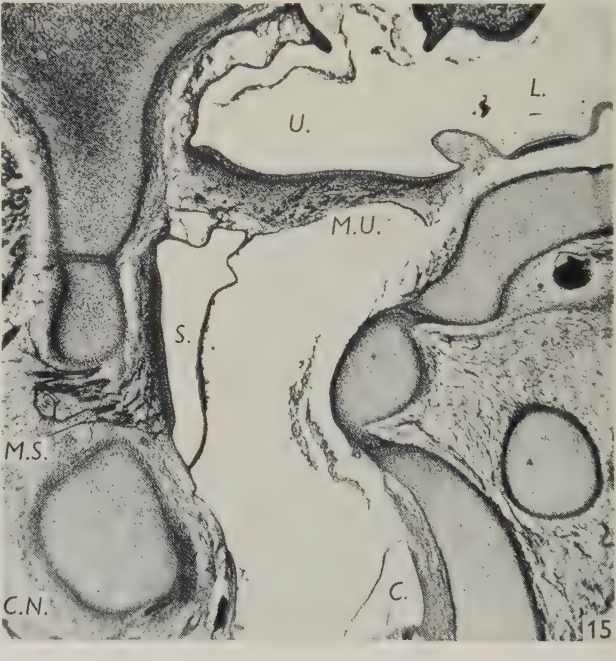

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SHUTE—Growth of neuro-epithelia in the human labyrinth

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Right ear of 17·5 mm. embryo. Transverse section showing lateral cristal nerves, lateral crista continuous with undifferentiated epithelium of utricle, and superior utricular nerve. × 80.

Fig. 2. Right ear of 30 mm. embryo. Transverse section showing lateral crista and its nerve, utricular macula cut tangentially and the superior and main utricular nerves, undifferentiated epithelium of saccule and superior saccular nerve. × 80.

Fig. 3. Left ear of 43 mm. embryo. Coronal section showing portions of superior crista cut transversely and of lateral crista cut longitudinally, utricular macula, saccule with portion of epithelial diverticulum, and cochlear duct. × 80.

Fig. 4. Left ear of 30 mm. embryo. Transverse section showing main saccular nerve and saccular macula continuous with undifferentiated epithelium of cochlear duct. × 80.

Fig. 5. Right ear of 43 mm. embryo. Sagittal section showing portion of basal coil of cochlear duct, subepithelial cochlear nerve fibres, neuro-epithelium at an early stage of differentiation, and a *fibre égarée*. × 400.

Fig. 6. Right ear of 62 mm. embryo. Coronal section showing neuro-epithelium of basal coil of cochlear duct, intra-epithelial nerve fibres to primordial inner and outer hair cells, tectorial membrane and vas spirale. × 400.

Fig. 7. Right ear of 48 mm. embryo. Sagittal section showing ductus reuniens, commencement of cochlear duct with epithelial diverticulum cut transversely, and cochlear nerve fibres. × 80.

Fig. 8. Left ear of 43 mm. embryo. Coronal section showing contribution of superior utricular nerve to utricular macula with adjacent undifferentiated epithelium, and undifferentiated epithelium of saccule intervening between portions of macula supplied by superior and main saccular nerves. × 80.

Fig. 9. Right ear of 62 mm. embryo. Coronal section showing undifferentiated epithelium of apex of cochlear duct, and subepithelial nerve plexus. × 80.

PLATE 2

Fig. 10. Right ear of 92 mm. embryo. Sagittal section showing organ of Corti of apical coil, and *fibre égarée* with its cone of growth at surface of epithelium. × 400.

Fig. 11. Right ear of 92 mm. embryo. Sagittal section showing neuro-epithelium in lagenar region of cochlear duct. × 400.

Fig. 12. Left ear of 92 mm. embryo. Coronal section showing superior (lateral, since rotation of utricle) and main utricular nerves entering anterior end of utricular macula, which, being upturned, is cut obliquely. × 80.

Fig. 13. Right ear of 92 mm. embryo. Sagittal section showing cubical epithelium of ductus reuniens, continuous with undifferentiated epithelium of commencement of cochlear duct, and subepithelial cochlear nerve fibres. × 400.

Fig. 14. Left ear of 92 mm. embryo. Coronal section showing portion of saccular macula, with thick fibre ending in calyx and giving off thinner branch. The neuro-epithelium is less densely innervated towards the left (= the upper part of the macula). × 400.

Fig. 15. Left ear of 92 mm. embryo. Coronal section showing portions of superior and lateral cristae, utricular nerve bundles underlying lateral part of utricular macula, saccular nerve fibres entering lower part of saccular macula, and basal coil of cochlear duct. × 30.

Key to lettering

C.	Cochlear duct	*M.U.*	Main utricular nerve
C.A.	Common ampullary nerve	*R.*	Ductus reuniens
C.N.	Cochlear nerve	*S.*	Saccule
D.	Epithelial diverticulum	*S.A.*	Superior ampullary nerve
L.	Lateral ampulla	*S.S.*	Superior saccular nerve
L.A.	Lateral ampullary nerve	*S.U.*	Superior utricular nerve
L.T.	Lower trunk of main utricular nerve	*U.*	Utricle
M.S.	Main saccular nerve		

A CYTOLOGICAL AND HISTOCHEMICAL STUDY OF BONE AND CARTILAGE FORMATION IN THE RAT

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INTRODUCTION

Recent advances in histochemical and cytological techniques have given a fresh impetus to the study of cell morphology in relation to functional activity. So far, however, comparatively little attention has been given to the bone-forming cells in this respect, and it was therefore decided to investigate certain aspects of the cytology of these cells in early centres of ossification in the hope of throwing light on the significance of the morphological changes which accompany the differentiation of osteoblasts from their mesenchymal precursors, and to attempt to correlate these changes with stages in the deposition of bone matrix. Similar, but less detailed, investigations were also carried out on the chondroblastic series of cells.

The investigation has been made on material from rat foetuses, and special attention was given to the distribution of alkaline phosphatase, glycogen, nucleoproteins, mucopolysaccharides, mitochondria and Golgi elements.

The association of alkaline phosphatase with the process of calcification in bone, cartilage and tooth has been confirmed by numerous workers since Robison (1923) first suggested it. Since Gomori (1939) published his well-known histochemical method a number of investigators have studied phosphatase activity in these tissues at the cytological level, and it is now established that osteoblasts, hypertrophic cartilage cells and odontoblasts show a high degree of enzyme activity. Variations in phosphatase activity during the differentiation of osteoblasts, and at different stages in matrix deposition, however, do not appear to have been studied, so that it is an open question whether phosphatase activity is solely, or even principally, associated with calcification, or whether it is in some way related also to the processes of cell differentiation and the deposition of the organic matrix.

The presence of glycogen in calcifying tissues has long been known, but received little attention until Harris (1932) first suggested that it might be the chemical precursor of the hexosephosphate esters postulated by Robison as the substrate for alkaline phosphatase in the calcification process. The discovery by Gutman & Gutman (1941) of a phosphorylase in calcifying cartilage which could convert glycogen into hexosephosphate, and other biochemical studies of recent years, have tended to support Harris's hypothesis. Our knowledge of the precise distribution of glycogen in bone and cartilage, however, is meagre, and as in the case of alkaline phosphatase, it is not known whether glycogen storage in these tissues is solely related to the process of calcification, or whether it has other roles.

The investigations of Brachet (1947, 1950), Caspersson (1947) and others in recent years have resulted in the identification of basophilic substances in many types of cell with nucleic acids, and the hypothesis that these are concerned in the manufacture of proteins is strongly supported. In view of this it seemed desirable to study afresh the well-known cytoplasmic basophilia of the osteoblast, for protein manufacture may well be an important function of this cell in connexion with the deposition of the organic components of the bone matrix. The chondroblast, likewise, seems implicated in the deposition of cartilage matrix, and its basophilia has also been investigated.

Young connective tissues show metachromasia to basic dyes, and recolour Schiff's reagent after periodic acid, owing to their content of mucopolysaccharides. The well-known metachromasia of cartilage matrix is also attributable to its mucopolysaccharide content. The importance of these substances in connective tissue economy is now being realized (Gersh & Catchpole, 1949), and it was therefore decided to investigate their distribution in developing bone matrix.

Finally, it is generally agreed that the osteoblast possesses a large Golgi element (Fell, 1925; Hill 1936) and numerous mitochondria (Dubreuil, 1913), but little is known about their behaviour during the differentiation of the cell and at different phases of its functional activity. In view of recent advances in our knowledge of the structure and chemical composition of these organelles in other cells, it seemed desirable to re-investigate them in the osteogenic series of cells.

MATERIALS AND METHODS

Norwegian rat foetuses varying in age from 12 to 20 days were obtained from animals whose pregnancies were timed from the day of finding spermatazoa in the vaginal smear.

Generally the foetuses were decapitated and the heads and trunks fixed separately, partly because most of the observations were to be made on the head, and partly because decapitation assisted the penetration of the fixatives.

A variety of fixatives was used according to the staining methods subsequently to be employed, including Bouin's and Helly's fluids, Regaud's and Aoyama's fixatives, and absolute alcohol. The specimens were not decalcified.

All embedding was done in paraffin, and sections were cut 2–7 μ thick according to the type of observation to be made on them.

Glycogen was usually demonstrated after fixation by absolute alcohol; Bouin's and Helly's fluids were also tried, but were found to be unsatisfactory owing to partial loss and diffusion of the carbohydrate. Sections 5–7 μ thick were flattened on 70 % alcohol and dried with a minimum of heat. After removal of wax they were treated with a thin (0·5 %) solution of celloidin in alcohol-ether. A number of staining methods were used. Best's carmine, and iodine, were found to be unreliable, so most sections were stained by either Hotchkiss's (1948) periodic acid-Schiff (P.A.S.) method, or by the author's chromic acid-diammino silver technique (Pritchard, 1949). These two methods gave comparable results, except that the smaller particles of glycogen were more sharply defined by the silver method. A saliva control test was performed on each batch of sections.

Alkaline phosphatase activity was investigated after absolute alcohol fixation by Gomori's method, using a veronal buffer. In order to reduce diffusion of the enzyme and its products to a minimum, sections were treated as for glycogen above, and the incubation times were kept as short as possible. Thus incubation times of 10 min., 30 min., 1 hr., and 2 hr. were employed as a routine on each batch of sections, and most of the significant observations were made after the shorter periods. Controls were obtained by omitting the glycerophosphate from the incubating solution; these showed the distribution of preformed inorganic phosphates in the bone matrix. In some cases another type of control was employed. Sections were dipped into either 5 % trichloracetic acid or hot (80° C.) water for 5–10 min. before incubating in the usual way. The enzyme was destroyed by these procedures.

Cytoplasmic basophilia was usually demonstrated by pyronin-methyl green staining. In correct proportions this combination stained the basophilic material of the cytoplasm, and the centre of the true nucleolus (plasmosome), red and that of the nucleus, including the periphery of the nucleolus, bluish green. The proportions needed had to be found by trial, as they varied according to the fixative. It was found that the clearest-cut results were obtained after absolute alcohol fixation, although Helly's and Regaud's fluids gave reasonably satisfactory pictures. Other basic dyes such as methylene blue, toluidine blue, thionin and saffranin were also used, but these did not differentiate between cytoplasmic and nuclear basophilia so clearly as did the pyronin-methyl green mixture. In some cases control sections were digested with a ribonuclease preparation in order to determine how far the cytoplasmic basophilia shown by pyronin was due to ribonucleic acids.

The *desoxyribonucleic acids* of the nucleus were stained by Feulgen's method, after fixation by absolute alcohol, Helly's or Regaud's fluids. *Mucopolysaccharides* were identified by their metachromatic reaction towards basic dyes, especially toluidine blue, and by their ability to recolour Schiff's reagent after periodic acid (P.A.S. test), it being generally agreed that few substances apart from mucopolysaccharides give both these reactions.

For the study of *mitochondria* the tissues were fixed in Regaud's or Helly's fluid, post-chromed, and stained with either Heidenhain's iron haematoxylin, Altmann's aniline-fuchsin-methyl green, Sudan black, or by the author's silver-reduction method (Pritchard, 1952). This last method consists essentially in placing sections, from distilled water, for 10–20 sec. in diluted Wilder's silver diammino hydroxide solution, and without washing plunging them with agitation into a large volume of very dilute formalin (0·1 %) for 20 sec., and finally washing in tap water. Usually some nuclear staining occurred, but this could be removed by the cautious application of 2 % potassium ferricyanide solution, differentiation being controlled under the microscope. The mitochondria were left sharply and densely stained against a clear background, except for calcified bone matrix which was also densely stained. It was usually desirable to counterstain with a red dye such as saffranin, acid fuchsin or eosin. Mitochondria were also demonstrated supravitally in smears of periosteal cells scraped from the surface of the foetal skull vault and stained with Janus green B (1/10,000).

The *Golgi element* was shown by Aoyama's cadmium-silver method. In osteoblasts, but not in other cells, the element was also often clearly visible in preparations stained for reticular fibres by Wilder's method after Bouin fixation. In some cases, again, the author's silver method for mitochondria after Helly's fixation showed the Golgi element clearly in a number of cell types, including the osteoblast.

Besides these more or less specific staining methods, the general histology of centres of ossification was studied in preparations stained by Delafield's, Weigert's and Heidenhain's haematoxylin, with eosin, Van Gieson, and Ponceau fuchsin-light green as counterstains.

In order to correlate the results obtained by different staining methods, wherever possible adjacent sections from a series were stained by different methods. Thus after alcohol fixation, glycogen, alkaline phosphatase, cytoplasmic basophilia and mucopolysaccharides were studied in successive sections: and after Helly's and Regaud's fixatives, mitochondria, basophilia, the Feulgen reaction and the general morphology were studied in a similar manner. The use of counterstains such as saffranin, toluidine blue and methylene blue, which give an overall picture of cytoplasmic and nuclear basophilia as well as matrix metachromasia, in preparations designed primarily to show glycogen, alkaline phosphatase, the Golgi element or the mitochondria, helped greatly in the task of correlating the various cytological characteristics one with another, and also with the stages in matrix deposition.

PRELIMINARY OBSERVATIONS

(Including terminology)

The observations on the cytology of the osteogenic series of cells described in this paper were made at sites of intra-membranous ossification in the skull vault, the facial skeleton and the periosteum of long bones. Endochondral ossification was not investigated. The cytology of the cartilage series of cells was studied in the chondrocranium, including Meckel's cartilage, and the cartilage models of the limb bones, ribs and vertebrae.

Preliminary observations by routine histological methods for the demonstration of cells and fibres were made on foetal rat, mouse, rabbit and human material with the object of providing a uniform terminology to serve as a basis for the subsequent cytological observations.

These observations showed that once the osseous territories had become defined by the differentiation of a fibrous periosteum, the periosteal surfaces of all actively growing bones, whatever their origin, presented a similar pattern of cell and fibre lamination (Pl. 1, figs. 1, 2, and 4). From without inwards the following zones could be identified: (1) a zone of *periosteal fibroblasts* and tangentially orientated coarse collagen fibres; (2) a zone of proliferating and differentiating *pre-osteoblasts* in a network of fine collagen fibres; (3) a zone of *definitive periosteal osteoblasts* arranged along radially directed bundles of coarse collagen fibres which merged into trabeculae of uncalcified bone matrix (osteoid) and then into calcified bone; and (4) a network of primary cancellous bone whose cavities were lined with *medullary osteoblasts*. In the earlier stages of osseous development all these zones were readily identifiable in transverse or longitudinal sections, but at later stages, owing to a reduction in the thickness of the inner zones, they were shown best in oblique tangential sections which exaggerated their thickness (cf. Stump, 1925).

Although the definitive periosteum of all the growing bones presented this uniform appearance, the method of arriving at this condition differed somewhat as between the bones of the cranial vault, the facial skeleton, and the bones of the axial skeleton and limbs. In the cranial vault the fibrous periosteum was differentiated simultaneously with the appearance of pre-osteoblasts at sites of imminent osteogenesis. In the facial skeleton (Pl. 1, fig. 5) condensations of pre-osteoblasts and the maturation of the more centrally placed of these into definitive osteoblasts, preceded the formation of the fibrous periosteum; while in the rest of the skeleton the fibrous perichondrium acted as a ready made fibrous periosteum once the underlying chondroblasts had disappeared and the pre-chondroblasts had been transformed into pre-osteoblasts.

The definitive periosteal osteoblasts (Pl. 1, fig. 3) were large pyriform or ovoid cells with an eccentrically placed hypochromatic nucleus containing one to three conspicuous nucleoli. Their cytoplasm appeared granular and was markedly basophilic except for a large clear area next to the nucleus termed the *juxta-nuclear vacuole.* Cytoplasmic processes united adjacent cells.

The *pre-osteoblasts* included an outer zone of small round cells with a centrally placed, moderately chromatic nucleus, small nucleoli and only slight cytoplasmic basophilia; and an inner zone of cells with morphological characteristics intermediate between those of the outer zone and definitive periosteal osteoblasts. Mitotic figures were frequent among the pre-osteoblasts, especially in the outer zone.

The *medullary osteoblasts* showed all transitions between cells with characteristics identical with those of the periosteal osteoblasts, and flattened inconspicuous resting cells lining inactive bony surfaces

Centres of chondrification also showed a gradation of cell and matrix types from without inwards (Pl. 1, fig. 6). The outermost zone of *perichondrial fibroblasts* and tangentially running coarse collagen fibres was followed by a zone of small round *pre-chondroblasts* in a network of fine collagen fibres, and then by a zone of *chondroblasts* in which the fibrous matrix was progressively masked by a deposit of hyaline cartilage matrix. This zone in turn merged into definitive cartilage containing *chondrocytes* and an abundance of hyaline matrix.

In certain cartilages, and at a later stage, hypertrophy of the chondrocytes was accompanied by a partial loss of matrix and the calcification of the remainder. This was followed by cellular degeneration and osteoblastic invasion preliminary to endochondral ossification.

In some situations, e.g. the ramus of the mandible, cartilage islands were formed within areas of membrane bone formation. This secondary cartilage (De Beer, 1937) appeared to arise by the differentiation of pre-osteoblasts into chondroblasts. Such cartilage was either completely resorbed, converted directly into osteoid, or else formed a growth cartilage to be replaced gradually by endochondral bone.

HISTOCHEMICAL AND CYTOLOGICAL OBSERVATIONS

Alkaline phosphatase (Pl. 3)

Bone formation

With prolonged incubation almost all nuclei will show enzyme activity, part of which is genuine and part of which is an artefact due to diffusion. For the purposes of this paper only phosphatase activity which is demonstrable in under 1 hr. is considered significant. Many of the observations were made after only 10 min. incubation in order to reduce artefacts due to diffusion to a minimum, and also to show up sites of maximal activity preferentially.

Enzyme activity appears coincidentally with the emergence of condensations of pre-osteoblastic mesenchyme, i.e. on the 14th day of pregnancy for the mandible, clavicle and maxilla, which are the earliest centres to appear. At this stage the nuclei of the pre-osteoblasts are sharply stained with the cobalt sulphide precipitate in contrast with the nuclei of the general mesenchyme which are unstained (Pl. 3, fig. 21). The cytoplasm and the intercellular fibrillar matrix are unstained at this stage. As the pre-osteoblasts *begin* to differentiate into definitive osteoblasts enzyme activity is greatly augmented as shown by the heavier precipitation (Pl. 3, fig. 22). Not only the nuclei, but the cytoplasm and intercellular fibres (Pl. 3, fig. 23) as well show intense activity. The definitive osteoblasts, however, while continuing to show intense activity, exhibit rather less activity than the layer of pre-osteoblasts which immediately surrounds them, their cytoplasm in particular being less heavily stained (Pl. 3, figs. 24, 25). The osteogenetic fibres between the cells are at first intensely active but they lose their activity as they become incorporated within the bone matrix. Similar extracellular phosphatase activity is always found where pre-osseous collagen fibres are being formed, whether it be in the periosteum, or in the medullary spaces.

The fibroblastic layer of the periosteum shows no activity except after prolonged incubation, when the *nuclei* begin to stain patchily near sites of intense activity in the deeper layers of the periosteum. This appearance must be interpreted as a diffusion artefact. The nuclear staining of the pre-osteoblasts, however, is unlikely to be an artefact, because it appears in the earliest mesenchymal condensations before activity has appeared in any other cells (Pl. 3, fig. 21). Similarly, the nuclear activity of the osteoblasts is probably genuine, for it is evident after very short periods of incubation and is unrelated to the intensity of staining of the neighbouring cytoplasm. After 2 hr. incubation, however, diffusion artefacts in the osteoblastic layer are very obvious in some sections, for the nuclei become intensely stained out of proportion to the increased staining of the cytoplasm.

With short periods of incubation, then, developing membrane bones show the following regional differences in enzyme activity (Pl. 3, figs. 23–25). The fibroblastic zone is inactive; the pre-osteoblastic zone shows at first only nuclear activity, followed by intense nuclear, cytoplasmic and extracellular activity; the periosteal osteoblasts show intense nuclear, less intense cytoplasmic and a diminished extracellular activity; while the medullary osteoblasts show similar features to the periosteal though less intensely, and the extracellular activity is confined to the surface of newly formed osteoid lamellae.

Similar differences in enzyme activity are found in the periosteum of the cartilage bones at the onset of ossification (Pl. 3, figs. 26, 27, 29). The differences are naturally most readily observed where growth is particularly rapid, and the cellular zones are thickest.

The phosphatase-positive osteogenetic tissue and the phosphatase-negative fibroblastic tissue are very clearly delineated at the sutural junctions (Pl. 3, fig. 28), where differentiation of osteoblasts is seen to be taking place on either side of a neutral non-osteogenetic zone.

The association of phosphatase activity with calcification is shown by comparing adjacent sections stained for phosphatase and for pre-formed bone salt, from which it is evident that phosphatase activity is intense in areas undergoing calcification. However, the greatest intensity is found in the region of pre-osteoblasts and pre-osseous collagen fibres just outside the region in which bone salt is being deposited.

Cartilage formation

Alkaline phosphatase activity is not found in association with the formation of cartilage unless hypertrophic changes supervene. Cartilages which do not ossify, such as some of the nasal cartilages and the greater part of Meckel's cartilage, therefore show no activity at any time.

The onset of the hypertrophic changes is marked by the appearance of nuclear phosphatase activity in the cells which are about to enlarge (Pl. 3, fig. 30). As the cells hypertrophy nuclear activity becomes much more intense and then the cytoplasm and finally the extra-cellular matrix show intense activity as well. The appearance of extracellular activity is associated topographically with the deposition of inorganic salts in the residual matrix. Like the inorganic salt, extracellular phosphatase activity first appears in the capsular zones surrounding the cells, and then spreads throughout the matrix. Eventually, as the hypertrophic cartilage cells degenerate, enzyme activity diminishes and finally disappears.

Glycogen (Pl. 2)

Bone formation

The pre-osteoblastic cells of the anlagen of the membrane bones show uniform and intense glycogen storage in their cytoplasm (Pl. 2, fig. 11). This is in sharp contrast with the surrounding non-osteogenetic mesenchyme which shows little or no glycogen except where cartilages and muscles are about to be formed. Comparison of adjacent sections stained for glycogen and phosphatase shows that the areas of intense glycogen storage correspond very well with the areas of nuclear phosphatase activity, though if anything the glycogen area is a little larger, indicating that glycogen storage slightly precedes phosphatase activity in the differentiation of pre-osteoblastic cells from the mesenchyme. Later the more centrally placed pre-osteoblasts show diminished glycogen storage as phosphatase activity becomes maximal (Pl. 2, fig. 12). Fully mature periosteal osteoblasts are characterized by their low glycogen content; in many places they appear to contain no glycogen at all (Pl. 2, figs. 13, 14 and 16). The medullary osteoblasts, on the other hand, usually contain some glycogen, but never as much as the pre-osteoblastic cells of the periosteum.

Glycogen is also found in the reticular cells of the bone marrow, and in some of the developing blood cells, but conditions were not favourable for identifying the cell types concerned.

This pattern of glycogen storage, viz. heavy deposition in the earlier pre-osteoblasts, dropping to little or none in the periosteal osteoblasts, with a reappearance of glycogen in the medullary osteoblasts, was a constant feature of all the membrane and cartilage bones studied. The fibroblastic zone of the periosteum, however, showed little or no glycogen storage at any time (in contrast to Bevelander & Johnson's (1950) findings). This was very apparent at the cranial sutures, where the sutural fibroblasts were clearly demarcated from the osteogenetic cells on either side by the difference in glycogen content (Pl. 2, figs. 19, 20), as they were by differences in alkaline phosphatase activity (Pl. 3, fig. 28).

Cartilage formation

In developing cartilage the pre-chondroblasts showed fairly intense glycogen storage, but no glycogen was found in the chondroblastic series of cells (Pl. 2, fig. 15). Only with the enlargement and approaching maturity of the cells and matrix did glycogen reappear (Pl. 2, fig. 17). The definitive chondrocytes invariably showed intense cytoplasmic storage. Like the fibrous periosteum, the fibrous perichondrium was glycogen-free. Glycogen storage remained intense in the early stages of cell hypertrophy prior to endochondral ossification, but the glycogen was lost as the matrix began to calcify and the cells degenerated (Pl. 2, fig. 18). Permanent hyaline cartilages retained intense glycogen storage indefinitely.

Both the bone and the cartilage-forming cells therefore agree in showing a sharp drop in glycogen storage at the onset of matrix deposition, but whereas in the bone-forming series of cells storage was highest at the pre-osteoblastic stage, in cartilage it was highest at the definitive chondrocyte change. The loss of glycogen from the cartilage cells as the matrix calcified was very striking. By contrast, in the bone-forming cells glycogen reappeared as the matrix became calcified.

In the course of their embryonic development many tissues exhibit either glycogen storage or alkaline phosphatase activity. With very few exceptions, however, the simultaneous presence of alkaline phosphatase activity *and* glycogen storage in the same cell is confined to the calcifying tissues (cf. Horowitz, 1942) though, as has been shown, in these tissues changes in glycogen storage and phosphatase activity do not run parallel courses.

Feulgen reaction for desoxyribose nucleic acids (Pl. 4, figs. 31, 32)

The nuclei of the pre-osteoblastic cells stained with about the same intensity as those of the general mesenchyme. As the nuclei enlarged and definitive osteoblasts appeared there was a progressive loss of staining intensity which persisted in the medullary osteoblasts (Pl. 4, fig. 32). The nuclei of osteoclasts and reticular cells, however, showed fairly intense staining, while those of the haemopoietic cells were very heavily stained. It was therefore an easy matter to distinguish osteoblastic nuclei from other nuclei in Feulgen preparations from a consideration of their staining intensity alone.

Mitotic figures, of course, show up clearly in Feulgen preparations, and it was at once evident that the majority of them lay in the outer pre-osteoblastic zone. Mitoses were not found in mature osteoblasts. This observation is in agreement with the widely held view that cell division is incompatible with mature functional activity.

The increase in nuclear size as osteoblasts were differentiated was accompanied by an increase in the size of the nucleoli also. The increase was apparently chiefly due to the increase in the ribonucleic acid component, for the larger nucleoli showed only a thin outer rim of Feulgen-positive material, the central part being unstained. The large nucleoli of the osteoclasts had a similar structure.

In the cartilage series of cells a similar, but even more striking, loss of Feulgen staining intensity was regularly observed (Pl. 4, fig. 31). The pre-chondroblasts stained with about the same intensity as the general run of mesenchymal cells, but the chondroblasts and chondrocytes showed very little staining at all.

These observations suggest a loss of desoxyribose nucleic acid from the nucleus during the differentiation of osteoblasts and chondroblasts, but the loss may be only an apparent one, a reflexion of the dilution of a constant amount of material as the nucleus enlarges, as has been maintained for other cells (Vendrely & Vendrely, 1949; Mirsky & Ris, 1947, 1949). The low staining intensity of the osteoblasts might be explained in this way, but it is difficult to believe that dilution alone accounts for the very dramatic loss of Feulgen intensity in the differentiating cartilage cells.

Cytoplasmic basophilia and ribonucleic acids

The intense cytoplasmic basophilia of the mature osteoblast has long been recognized. With any of the usual basic stains osteoblasts are always conspicuous because of their cytoplasmic basophilia (Pl. 4, fig. 34). Characteristically, the juxtanuclear vacuole remains clear (Pl. 1, fig. 3). With the pyronin-methyl green staining combination the osteoblast cytoplasm stained red and the nuclear membrane and chromatin granules blue-green. The nucleoli (plasmosomes), however, showed a central pyroninophil area and a peripheral rim of methyl-green stained material. The cytoplasmic basophilia of the osteoblasts (and indeed of all embryonic cells) was easily destroyed by hydrolysis with 1 % HCl at 60° C. for 2 min., and by the action of dilute alkalis in the cold, both procedures leaving the nuclear basophilia (except for the centre of the nucleolus) unaltered. Ribonuclease digestion also removed the basophilia of the cytoplasm and of the nucleolar centre.

Taking the above evidence, and that of the Feulgen studies, into consideration, there can be little doubt that the cytoplasmic and central nucleolar basophilia of the osteoblast is associated with its ribonucleic acid content. The basophilia was not uniform throughout the non-vacuolar cytoplasm, but appeared to be related to a granular material. Whether this was an artefact of fixation, or whether the ribonucleic acid was contained in preformed granular components of the cytoplasm, perhaps mitochondria and microsomes (cf. Brachet, 1947), could not be determined. The cytoplasm of the pre-osteoblasts showed a variable degree of basophilia, depending upon the stage of differentiation, but it was never so marked as in the definitive osteoblasts (Pl. 4, fig. 33).

The medullary osteoblasts showed less intense basophilia than the periosteal osteoblasts, the intensity varying with the size, and therefore presumably with the functional activity, of the cells. The osteocytes showed comparatively little basophilia, while the staining of the osteoclasts was very variable, in some the whole or part of the cytoplasm was intensely basophilic, in others the cytoplasm was eosinophilic.

The development of cytoplasmic basophilia in the cartilage series of cells ran an approximately parallel course to that of the osteoblastic series, increasing as the chondroblasts matured, and decreasing again in the mature chondrocytes. With the onset of hypertrophic changes cytoplasmic basophilia was lost. No cell of the cartilage series, however, ever showed such conspicuous cytoplasmic basophilia as did the periosteal osteoblasts, whose staining intensity was equalled only by the spinal ganglion cells and some of the epithelial cells of the developing skin, glands and alimentary tract.

In the osteogenetic series of cells maximum cytoplasmic basophilia, and therefore presumably maximum ribonucleic acid content, was associated with the sites of most rapid deposition of bone matrix. In the chondroblastic series also this relationship between basophilia and matrix deposition was observed, but in a less striking manner.

Mucopolysaccharides

Metachromasia towards toluidine blue and methylene blue was exhibited by the fibres and ground substance of the pre-osseous matrix surrounding the pre-osteoblasts of the periosteum, and by the newly deposited osteoid matrix adjacent to the definitive osteoblasts (cf. Follis & Berthrong, 1949; Bevelander & Johnson, 1950; Follis, 1951). Calcified bone matrix exhibited metachromasia only after treatment with dilute hydrochloric acid (cf. Howard, 1951*a*, *b*). With the periodic acid-Schiff test the pre-osseous fibres, osteoid and calcified matrix gave an increasingly strong positive reaction in that order.

Uncalcified cartilage matrix was of course intensely metachromatic but as in the case of bone, calcified cartilage matrix was only metachromatic after decalcification. Both calcified and uncalcified matrix gave a moderately strong positive reaction with the P.A.S. test.

This co-existence of metachromasia and a positive P.A.S. reaction in bone and cartilage matrices is presumptive evidence for the presence of mucopolysaccharides. It is not clear why the metachromatic reaction should be inhibited by calcium salts, but the appearance of the reaction after decalcification disposes of Sylvén's (1947*a*, *b*) contention that mucopolysaccharides disappear as calcification begins.

Mitochondria

Living osteoblasts scraped from the surface of the parietal bone of foetal rats and examined supravitally with 1/10,000 Janus green on a warm stage showed numerous filamentous mitochondria stained bright blue-green against a clear background. As the cells died the mitochondria fragmented into small granules and the nucleus and finally the general cytoplasm took up the stain. It would appear therefore that the mitochondria in the living osteoblast are filamentous in form, and that the granular forms sometimes found in stained sections are artefacts due to imperfect fixation (cf. Fischer, 1948). This was borne out in the specimens stained for mitochondria by the classical aniline-fuchsin, methyl-green technique, by iron haematoxylin and by the author's silver-reduction method, where, in the best-fixed preparations, and near the surface of specimens exhibiting poor fixation centrally, the mitochondria were uniformly filamentous or rod-like (Pl. 4, fig. 38), whereas in poorly fixed areas they were in the form of strings of granules, isolated granules or amorphous clumps. Mitochondria were most numerous in the large periosteal osteoblasts, in which they occupied every part of the cytoplasm except the juxtanuclear vacuole, and generally tended to be orientated in the long axis of the cell. A concentration of mitochondria around the vacuolar region was sometimes observed, but was never general (cf. Hill, 1936). Mitochondria in the fibroblastic and pre-osteoblastic zones of the periosteum were much less numerous (Pl. 4, fig. 36). In fact, under the lower powers of the microscope, the osteoblasts were easily identifiable by their mitochondrial content alone. The mitochondria of the medullary osteoblasts (Pl. 4, fig. 35), varied in numbers according to the size of the cell, but in general were less numerous than in the periosteal cells. They were filamentous in form in the best fixed preparations.

The osteoclasts were always striking in mitochondrial preparations because their cytoplasm was invariably packed with coarse granules and short rods (Pl. 4, fig. 37); filamentous forms were never observed so that it is unlikely that these appearances were artefacts.

In developing cartilage mitochondria were most numerous in the perichondrial cells. The mature cells usually contained granular forms rather than filaments (cf. Sheehan, 1948), but as the cartilage matrix is necessarily a barrier to rapid fixation, it is possible that the mitochondria are filamentous in the living cell.

In general, however, it may be said that in both the osteoblastic and chondroblastic series of cells, the mitochondria were most numerous where the rate of deposition of intercellular matrix was greatest.

The Golgi element

The true morphology of this organelle in living cells is still uncertain, but it is generally agreed that the structures seen in silver- and osmium-impregnated tissues are artefacts (myelin figures) produced during the precipitation of the lipoidal components. Nevertheless, the overall size and position of the precipitation artefact is a general guide to the size and position of the element in life, and these features can be regarded as affording evidence concerning the secretory activity of the cell.

In successful Aoyama preparations the periosteal cells show a localized deposit of silver next to the nucleus (Pl. 1, fig. 7) occupying the site of the juxta-nuclear vacuole seen in ordinary preparations (cf. Fell, 1925). The silver deposit is in the form of discrete granules in some cells (Pl. 1, fig. 8), in the form of a continuous reticulum in others (Pl. 1, fig. 9), but no significance can be attached to these morphological differences owing to the artificial character of the silver deposit. The size of the silver deposits increased steadily as the osteoblasts matured, those of the large periosteal osteoblasts having a diameter two to three times those of the early pre-osteoblastic cells (Pl. 1, fig. 10). which were in turn clearly larger than those of the periosteal fibroblasts, and the general mesenchyme.

With Aoyama's technique the cytoplasm of the medullary osteoblasts usually showed a uniformly heavy precipitate of silver throughout, and so the size and position of the Golgi element in these cells remained in doubt. It was found by accident, however, that after Bouin's fixation, Wilder's 'on-the-slide' silver method for demonstrating reticular fibres often showed a deposit of silver in the periosteal and medullary osteoblasts, strictly localized to the vacuolar area of the cytoplasm. No other cells showed this phenomenon. Also, after Helly's fixation, the author's silver method for mitochondria often showed discrete silver precipitation in the Golgi region of the cytoplasm of most of the cells in the embryo, including the osteogenetic series of cells. It therefore seems likely that the generalized argentophilia of the cytoplasm of the medullary osteoblasts shown by the Aoyama preparations does not give a true indication of the size of the Golgi element in these cells. More probably the Golgi element remains a discrete juxta-nuclear body throughout the life of the osteoblast.

Attempts were made to demonstrate the Golgi element by means of Sudan black B, both in paraffin sections after Helly fixation, and in frozen sections of material fixed with formol-calcium, but without convincing results. The osteoblasts, however, were conspicuous in such preparations because of the diffuse, finely granular, sudanophilia of their cytoplasm, while the osteoclasts showed numerous heavily coloured particles like those seen in the mitochondrial preparations already described.

The Golgi element of the chondroblastic series of cells was usually readily impregnated by Aoyama's technique. It took the form of a crescentic area of silver precipitation capping the nucleus. Unlike the osteogenetic series of cells, changes in the size of the element during the maturation of the chondroblasts were not found. There appeared to be a disintegration of the element at the hypertrophic stage.

DISCUSSION

The cytological and histochemical evidence presented in this paper amply justifies the classification of periosteal cells into three main groups, viz. fibroblasts, pre-osteoblasts and definitive osteoblasts.

The *definitive periosteal osteoblasts* are large pyriform or ovoid cells with an eccentric nucleus and a large juxta-nuclear vacuole. They tend to be arranged in columns and rows adjacent to newly formed bone matrix. They exhibit no mitoses, contain little or no glycogen, and their Feulgen-staining intensity is low. The cytoplasm of these osteoblasts is intensely basophilic, and both nucleus and cytoplasm show considerable alkaline phosphatase activity. The Golgi element is very large and there are numerous filamentous mitochondria. The associated intercellular matrix consists of bundles of collagen fibres which exhibit metachromasia to basic dyes and a high phosphatase activity, merging into trabeculae of osteoid bone matrix which also exhibit metachromasia, but no phosphatase activity, and then into trabeculae of definitive calcified matrix which only exhibit metachromasia after decalcification. Pre-osseous fibres, osteoid and calcified matrix give an increasingly intense positive reaction with the periodic acid-Schiff test.

The *pre-osteoblasts* represent the immature stages of osteoblastic development. They include (1) small, rounded, closely packed elements with a high mitotic rate, high Feulgen-staining intensity, conspicuous glycogen content but with phosphatase activity confined to the nucleus, as well as (2) a series of cell types approaching the definitive osteoblastic condition with fewer mitoses, reduced glycogen storage, a very high nuclear and cytoplasmic phosphatase activity and increasing cytoplasmic basophilia allied with decreasing intensity of Feulgen staining of the nucleus, and an increasing size of the Golgi element. The pre-osteoblasts as a group contain comparatively few mitochondria. The associated intercellular matrix appears oedematous, with moderate numbers of fine fibres which are argyrophilic, metachromatic, give a positive reaction with the periodic-Schiff test and show conspicuous phosphatase activity.

The medullary osteoblasts vary considerably in size. The larger ones show characteristics almost as pronounced as those of the definitive periosteal osteoblasts. The smaller ones show them in lessened degree.

The interpetation of these facts cannot be finally decided in the present stage of our knowledge, but there are a number of suggestive relationships:

(1) The overall cell enlargement, and increasing eccentricity of the nucleus during osteoblastic differentiation are easily explained as being due to the accumulation of cytoplasmic products: basophilic material (ribonucleoprotein), mitochondria, and the large Golgi element.

(2) There seems little doubt that the juxta-nuclear vacuole is occupied by the Golgi element (in agreement with Fell, 1925).

(3) The decline in mitotic activity is what might be expected in view of the generally observed inverse relationship between cell reproduction and functional activity.

(4) The decline in Feulgen-staining intensity may represent a real or an apparent loss of desoxyribose nucleic acid. In the former case it would agree with the decline in mitotic activity. On the other hand, the increasing nuclear size may be due to invisible chromosome reproduction, as has been found in other nuclei (Salvatore, 1950), associated with the dilution of a constant amount of nucleic acid.

(5) The great increase in cytoplasmic basophilia (ribonucleic acid) and the associated increase in nucleolar size are diagnostic of a cell actively engaged in protein manufacture (Caspersson, 1947). This evidence, taken in conjunction with the simultaneous increase in the intercellular matrix, particularly of its collagenous (protein) component, surely indicates that the osteoblast is actively secreting the protein basis of the matrix (cf. Cappellin, 1948). This view is further supported by the great rise in alkaline phosphatase activity which accompanies the signs of protein manufacture mentioned, for it is probable that the formation of fibrous proteins involves alkaline phosphatase activity (Bradfield, 1949, 1950; Jeener, 1947).

(6) The increase in mitochondria and the hypertrophy of the Golgi element support the thesis that the osteoblast is metabolically very active.

(7) Besides the possible significance of alkaline phosphatase in fibrous-protein manufacture alluded to, alkaline phosphatase on the evidence of this paper may well play a part: (*a*) in the intrinsic metabolism of cell differentiation, for nuclear phosphatase activity has been so interpreted by several workers (cf. e.g. Moog, 1944), (*b*) in the manufacture of cytoplasmic ribonucleic acid (Bradfield, 1950); and (3) in the mechanism of calcification.

Of course phosphatase may be concerned in all the processes mentioned, but at present its role in the mechanism of calcification is best supported by independent evidence, especially when its activity is reviewed in conjunction with the metabolism of glycogen in calcifying tissues. Thus the Gutmans' (1941) discovery of a phosphorylase as well as phosphatase and glycogen in calcifying cartilage prompted the hypothesis that the initial step in calcification is the hydrolysis of glycogen by phosphorylase to produce hexosephosphate esters which are in turn hydrolysed by phosphatase to liberate PO_4 ions. Then according to Roche & Mourgue (1943) and Roche & Deltour (1943) the PO_4 ions are temporarily fixed to the pre-osseous matrix, only to be liberated again with the formation of the definitive organic matrix, when they combine with calcium ions to form the inorganic matrix. It will be seen that part of the story is derived from the study of calcifying cartilage and part from the investigation of bone formation, but there is no reason to suppose that the mechanisms are very different. Roche's hypothesis explains one formerly puzzling feature of phosphatase histochemistry, namely the observation that enzyme activity is most intense at the pre-osseous stage *before* calcification has begun.

(8) The presence of glycogen in the precalcification stages of bone and cartilage development, and its abrupt disappearance with the actual onset of calcification, seem satisfactorily explained on the above theory, but there are other possible roles for the carbohydrate. Thus both in bone and cartilage, its temporary disappearance from the osteoblast and chondroblast is associated with the appearance of mucopolysaccharides in the intercellular matrices, and it is therefore possible that the breakdown of glycogen is associated with the manufacture of the mucopolysaccharides. However, we know too little about the formation of these substances to pursue this speculation further.

Then again glycogen may be held in the pre-osteoblastic and pre-hypertrophic cartilage cells as a store of potential energy to be used in the intense metabolic processes accompanying cell differentiation and secretion. Or again, as Harris (1933) suggests, glycogen storage may simply be indicative of relatively anaerobic conditions, and it is certainly true that cartilage, which contains much glycogen, is avascular, while the glycogen content of the osteoblastic series of cells seems to fall as the blood supply improves.

The changes undergone by the *cartilage* cell in its maturation from the mesenchyme show several features in common with the osteoblastic series of cells. On the morphological and histochemical evidence it seems justifiable to differentiate the following stages: *pre-chondroblastic* mesenchymal cells, *chondroblasts*, *chondrocytes*, and *hypertrophic chondrocytes*. The chief cytological trends observed during this maturation were: (1) A progressive increase in cell size. (2) A progressive decrease in Feulgen-staining intensity. (3) A very early period of glycogen storage at the pre-chondroblastic, followed by its complete absence at the chondroblastic stage; its reappearance and increasing deposition at the chondrocyte and early hypertrophic stages; followed by its disappearance as the matrix becomes calcified. (4) Phosphatase activity was absent from the early stages and did not appear unless hypertrophic changes supervened. Then it increased rapidly, first in the nucleus, then in the cytoplasm and finally extracellularly as the matrix calcified. (5) Cytoplasmic basophilia steadily increased during the chondroblastic stage of differentiation, but diminished again at the chondrocyte stage. (6) Changes in the Golgi element and mitochondria were not marked.

On the above evidence the participation of glycogen and alkaline phosphatase in the calcification mechanism in hypertrophic cartilage seems likely. The histochemical evidence, however, gives little indication of the way in which the *organic* matrix of cartilage is formed, or of the cellular role therein.

At this stage in our knowledge of bone formation the statement of a working hypothesis about the mechanism of the formation of the organic and inorganic matrices, and the role of the osteoblasts therein, taking into account the cytological, histochemical and chemical evidence, may be of some value. I would suggest therefore that the osteoblast secretes (1) alkaline phosphatase, (2) a collagen precursor, (3) hexose-phosphoric esters, (4) mucopolysaccharides; that these secretory products constitute the 'pre-osseous substance' of the French School; that the presence of mucopolysaccharides explains the oedematous appearance of the pre-osseous substance owing to their marked water-binding properties (Heringa, 1949); that the collagen is at first in an unaggregated, non-micellar, molecular form; that the hexose phosphates derived from the breakdown of glycogen (Harris, 1932; Gutman & Gutman, 1941) are hydrolysed by extracellular alkaline phosphatase (Robison, 1923) to provide a local excess of PO_4 ions which are at first fixed to the protein matrix (Roche & Deltour, 1943). Possibly also calcium ions are fixed independently to the mucopolysaccharides. Then there is a rather sudden physicochemical change in the state of aggregation of the collagen and mucopolysaccharide molecules which results in the precipitation of the osteoid matrix, while the calcium and PO_4 ions, liberated from their attachments, combine and precipitate to form the mineral salt (Roche & Deltour, 1943). Later other ions are added to give the mineral its complex structure (Dallemagne, 1943). This hypothesis accounts satisfactorily for most of the evidence we possess about the mechanism of osteogenesis. One difficulty remains, however, for in some situations (Weidenreich, 1928) bone formation apparently occurs with a direct transformation of fibroblast-like cells into osteocytes without the intervention of an osteoblast stage. I feel it would be worth while investigating this atypical method of ossification in order to see whether or not the cells involved show characteristic signs of osteoblastic activity such as intense cytoplasmic basophilia and a high phosphatase content, even if the typical osteoblast morphology is not reproduced in all its features. At any rate, the time is past when one can afford to neglect the cells, as Leriche & Policard (1928) have done, in any consideration of the mechanism of bone formation.

SUMMARY

1. A cytological and histochemical investigation of the processes of ossification and chondrification has been made on foetal rats with the objects of defining the changes which occur during the differentiation of osteoblasts and chondroblasts from their mesenchymal precursors, and of relating these changes to the stages in the deposition of the intercellular matrices.

2. Particular attention was paid to the changes in glycogen storage, alkaline phosphatase activity, cytoplasmic basophilia, the intensity of the Feulgen nuclear reaction, the number of mitochondria and the size of the Golgi element.

3. The cells involved in ossification were classified as periosteal fibroblasts, pre-osteoblasts, definitive periosteal osteoblasts and medullary osteoblasts; and those involved in chondrification as perichondrial fibroblasts, pre-chondroblasts, chondroblasts, non-hypertrophic chondrocytes and hypertrophic chondrocytes.

4. Glycogen storage was negligible in periosteal fibroblasts, intense in pre-osteoblasts, absent or negligible in definitive periosteal osteoblasts and moderate in medullary osteoblasts. It was negligible in perichondrial fibroblasts, intense in pre-chondroblasts, absent in chondroblasts, intense again in non-hypertrophic chondrocytes and greatly diminished in the later hypertrophic chondrocytes.

5. Alkaline phosphatase activity was absent from periosteal fibroblasts, entirely nuclear in early pre-osteoblasts, intense in nucleus, cytoplasm and intercellular fibres at the late preosteoblast stage and of diminished intensity in the nucleus, cytoplasm and fibres at the definitive osteoblast stage. In cartilage enzyme activity was restricted to the hypertrophic stage when it appeared first in the nucleus, then in the cytoplasm and finally in the extracellular matrix at the onset of calcification.

6. Cytoplasmic basophilia, shown to be due to ribonucleic acids, was moderate in periosteal fibroblasts and pre-osteoblasts but intense at the definitive osteoblast stage. It was less intense in medullary osteoblasts. Cytoplasmic basophilia increased in the cartilage series of cells up to the chondrocyte stage when a reduction in intensity was observed.

7. The intensity of the Feulgen nuclear reaction decreased markedly as osteoblasts and chondroblasts were differentiated.

8. Filamentous and rod-like mitochondria were very numerous in periosteal osteoblasts, less numerous in medullary osteoblasts and few in pre-osteoblasts and fibroblasts. They were most numerous at the chondroblast stage in the cartilage cell series.

9. The size of the Golgi element increased markedly as osteoblasts were differentiated from their mesenchymal precursors. The size and position of the element coincided with the size and position of the juxta-nuclear vacuole. The Golgi element showed no conspicuous changes in form and position during the differentiation of the cartilage cells.

10. The intercellular matrix of the fibroblastic layer of the periosteum contained comparatively stout collagen fibres orientated tangentially. That of the pre-osteoblastic layer contained a reticulum of fine fibres which exhibited metachromasia to basic dyes, a positive reaction to the periodic acid-Schiff test and intense alkaline phosphatase activity. That of the osteoblastic layer consisted of sheaves of collagen fibres merging into trabeculae of hyaline osteoid matrix and then into calcified bone. The fibres exhibited phosphatase activity but the osteoid did not. Fibres, osteoid and calcified bone (after decalcification) were all metachromatic and gave a positive periodic acid-Schiff reaction.

11. The evidence warrants the assumption that the osteoblast is an actively secreting cell.

12. These histochemical and cytological findings were discussed in the light of current theories about the biochemical mechanisms involved in bone formation and it was suggested that the osteoblast contributes to the deposition of the matrix by secreting (*a*) alkaline phosphatase, (*b*) a collagen precursor, (*c*) hexose phosphates and (*d*) mucopolysaccharides.

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EXPLANATION OF PLATES

All the illustrations shown were prepared from sections of foetal rat material with the exception of Pl. 1, fig. 3, which was from bony callus after fracture in an adult rat.

Plate 1 (general morphology and Golgi element)

Fig. 1. Periosteum of neck of mandible (18-day). Note zoning of cells and cytoplasmic basophilia of the deeper-lying osteoblasts. Haematoxylin and eosin. ×425.

Fig. 2. Periosteum of neck of mandible (18-day). Note tangential fibres of the outer layer of the periosteum above and radially orientated pre-osseous fibres below. Wilder. ×425.

Fig. 3. Row of osteoblasts against newly formed bone matrix (12-day callus). Note eccentricity of nucleus, large juxta-nuclear vacuole and cytoplasmic basophilia. Methylene blue and eosin. ×665.

Fig. 4. Periosteum and newly formed bone matrix from mandible (16-day). Note successive fibre zones; tangential above, then reticular, then radial pre-osseous fibres below passing into coarse strands of definitive bone matrix. Wilder. ×540.

Fig. 5. Edge of mandibular ossification centre (16-day). Note central osteoblasts surrounded by pre-osteoblasts. Haematoxylin and eosin. ×425.

Fig. 6. Perichondrium of metacarpal (16-day). Note successive zones of fibroblasts, pre-chondroblasts and chondroblasts from above downwards. Haematoxylin and eosin. ×540.

Fig. 7. Periosteal osteoblasts from the mandible (20-day). Note the conspicuous Golgi elements adjacent to the nucleus. Aoyama. ×540.

Fig. 8. Periosteal osteoblasts from the mandible (20-day). Note the granular character of the Golgi element in this preparation. Aoyama. ×1665.

Fig. 9. Osteoblasts from the parietal bone (18-day). Note the reticular character of the Golgi element in the central cell. Aoyama. ×1665.

Fig. 10. Part of the skull vault near the sagittal suture (18-day). Note the large size of the Golgi elements of the centrally placed osteoblasts compared with those of the more peripherally placed pre-osteoblasts and fibroblasts. Aoyama. ×200.

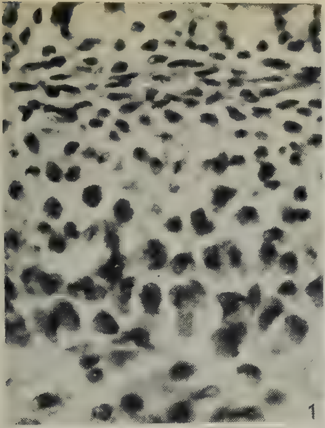
Plate 2 (glycogen)

Fig. 11. Glycogen of mesenchymal anlagen of mandible and Meckel's cartilage (14·day). Clear oval area marks position of the inferior dental nerve. P.A.S. ×130.

Fig. 12. Glycogen of mandible and Meckel's cartilage (15 day). Note decreased glycogen storage in the central area of differentiating osteoblasts compared with the surrounding pre-osteoblasts. P.A.S. ×130.

Fig. 13. Glycogen of radius and surrounding musculature (16-day). Note glycogen in cartilage cells, pre-osteoblasts and muscles but not in the osteoblasts. Cf. Pl. 3, fig. 29, which shows alkaline phosphatase activity in an adjacent section. Author's silver method. ×100.

Fig. 14. Tangential section of edge of mandible (17-day). Note glycogen in the pre-osteoblasts and muscles but its absence from the fibroblastic and osteoblastic zones of the periosteum. Best's carmine and cobalt sulphide. ×130.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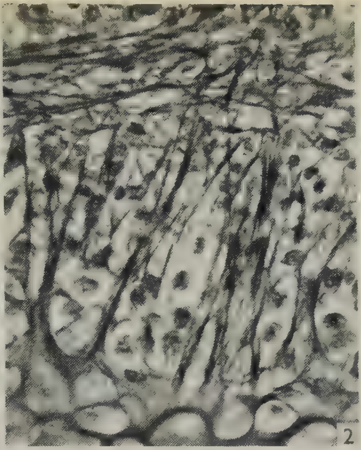
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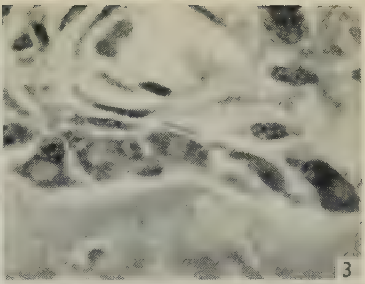
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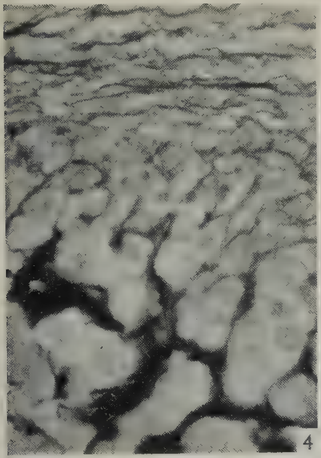
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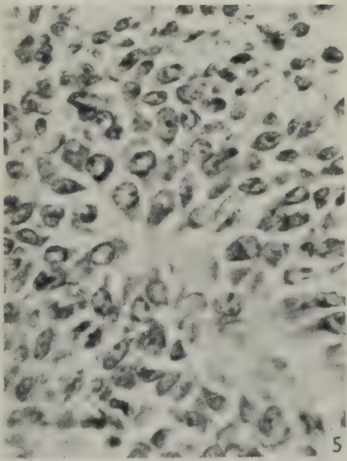
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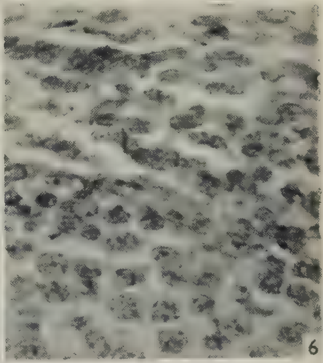
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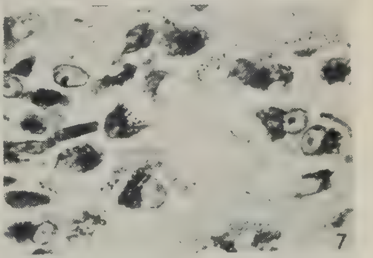
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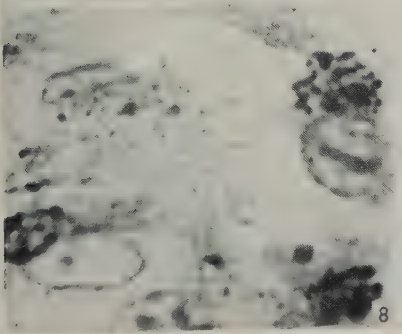
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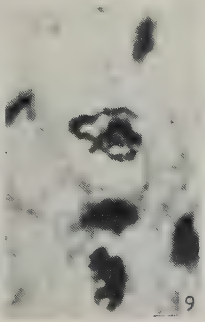
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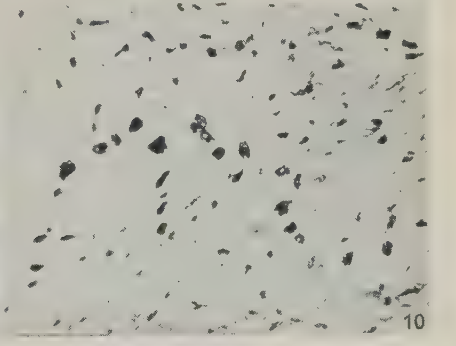
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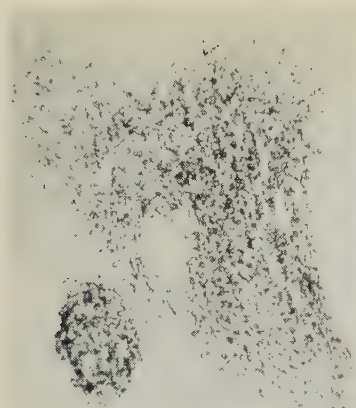
PRITCHARD—BONE AND CARTILAGE FORMATION IN THE RAT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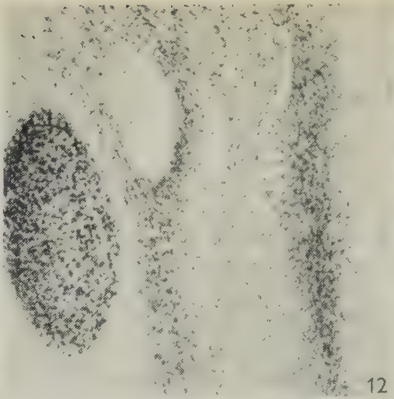
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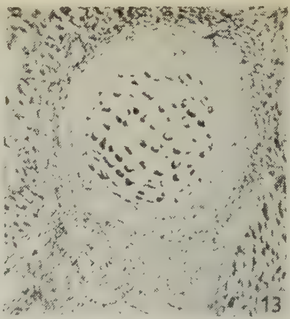
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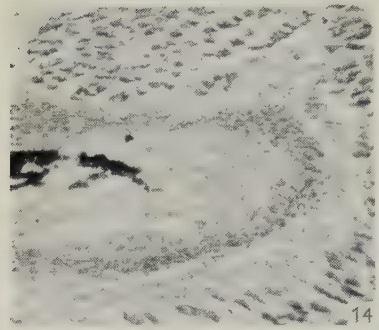
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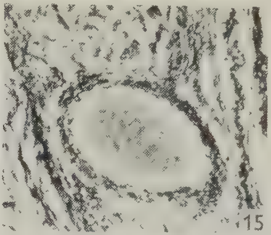
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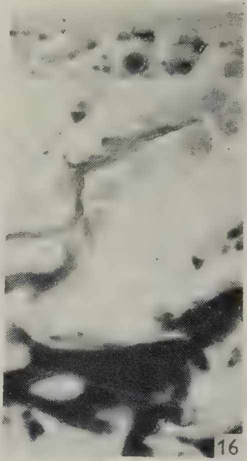
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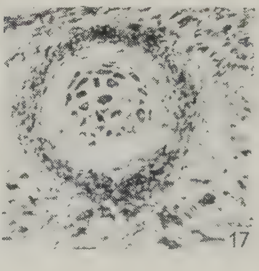
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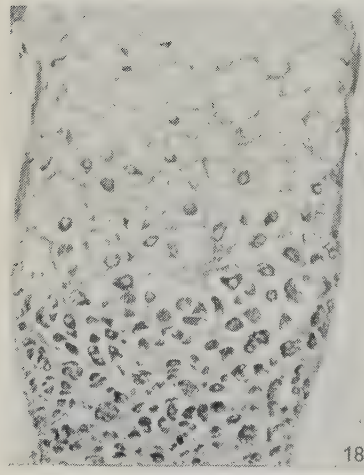
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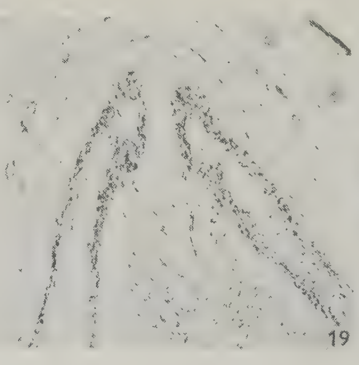
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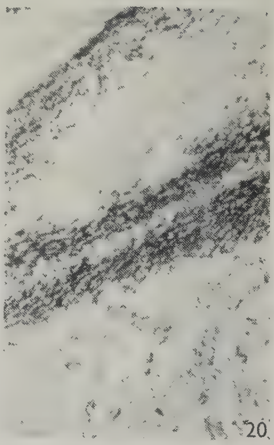
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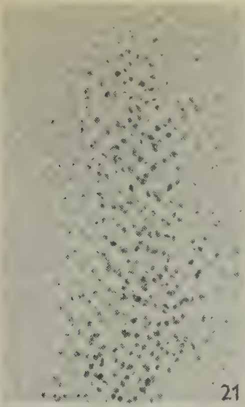
PRITCHARD—Bone and cartilage formation in the rat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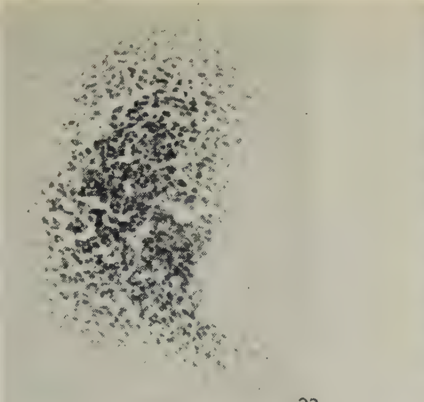
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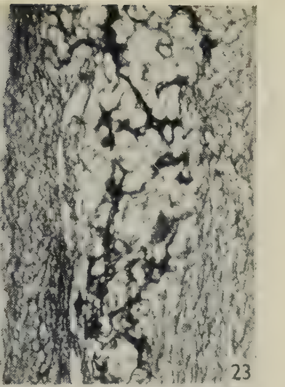
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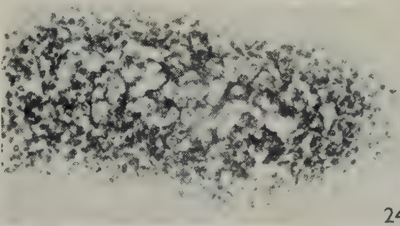
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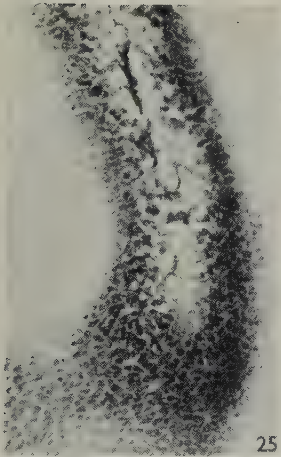
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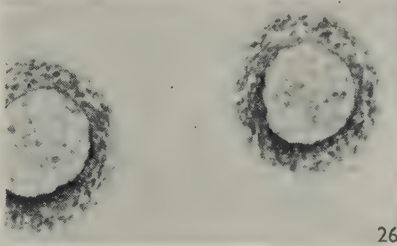
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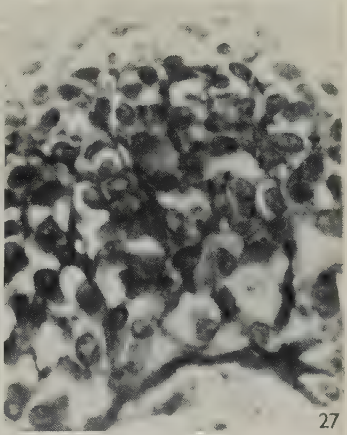
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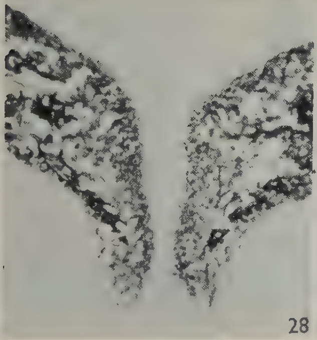
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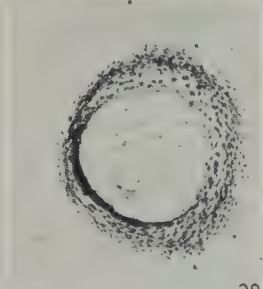
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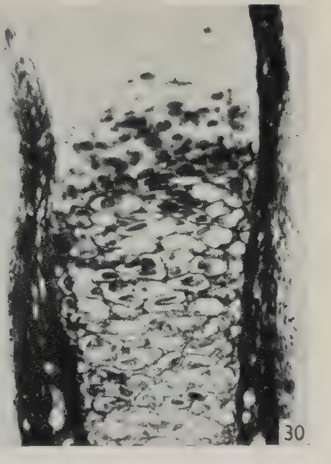
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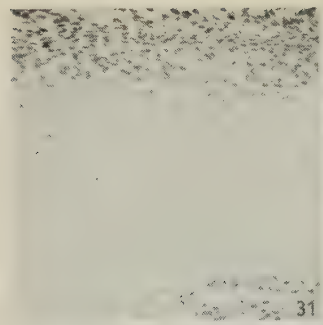
PRITCHARD—Bone and cartilage formation in the rat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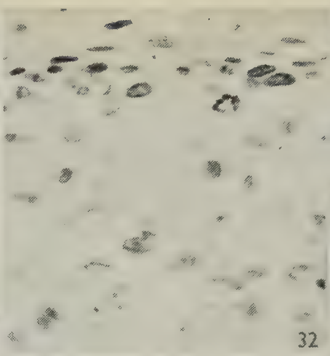
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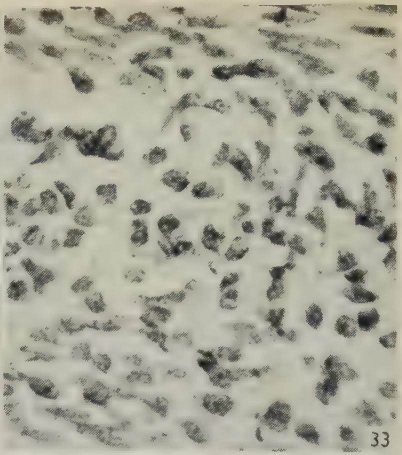
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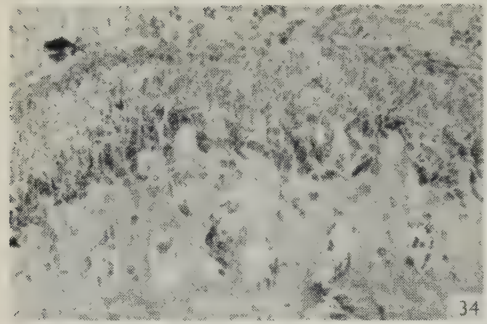
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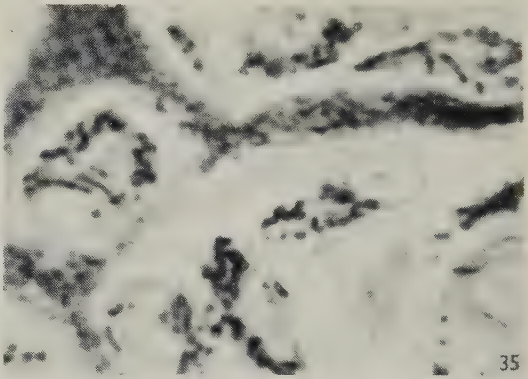
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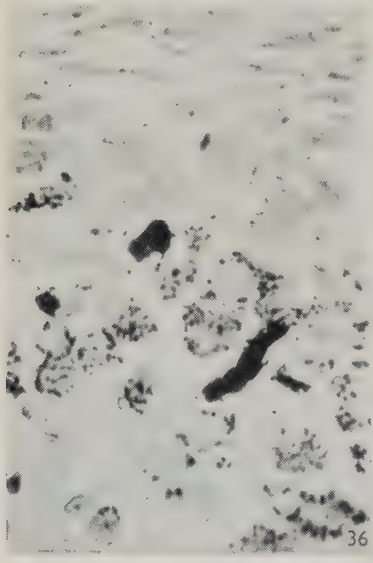
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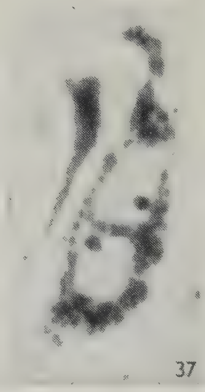
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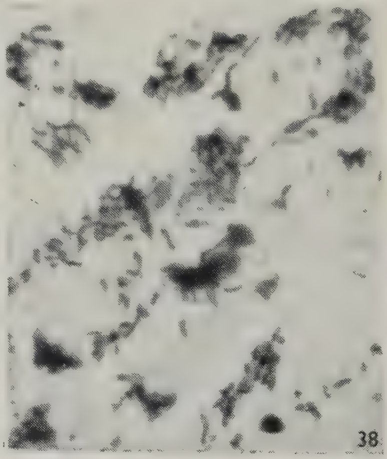
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PRITCHARD—Bone and cartilage formation in the rat

Fig. 15. Costal cartilage (16-day). Note presence of glycogen in the pre-chondroblastic zone but its absence from the chondroblasts. Author's silver method. × 130.

Fig. 16. Mandible (17-day). Glycogen present in the pre-osteoblasts above and the medullary osteoblasts below but absent from the column of periosteal osteoblasts. Best's carmine and cobalt sulphide. × 540.

Fig. 17. Rib (16-day). Glycogen in mature chondrocytes and pre-osteoblasts but none in the osteoblasts. Author's silver method. × 130.

Fig. 18. Basisphenoid (19-day). Note decrease in glycogen storage accompanying hypertrophic changes in the chondrocytes preparatory to endochondral ossification. P.A.S. × 130.

Fig. 19. Parietal part of sagittal suture (17-day). Glycogen present in pre-osteoblasts but not in the sutural fibroblasts. Author's silver method. × 130.

Fig. 20. Maxillary-premaxillary suture (19-day). Note heavy glycogen storage in the pre-osteoblasts on either side of the sutural fibroblasts. P.A.S. × 130.

PLATE 3 (alkaline phosphatase)

Fig. 21. Maxillary primordium (14-day). Note nuclear phosphatase of early pre-osteoblasts. × 130.

Fig. 22. Mandibular primordium (14-day). Note increased enzyme activity in the late pre-osteoblasts centrally. × 130.

Fig. 23. Mandible (16-day). Note intense enzyme activity of the fibres of the pre-osteoblastic zone. The central network of bone trabeculae is calcified. × 130.

Fig. 24. Clavicle (14-day). Note decreased phosphatase activity of the definitive osteoblasts centrally × 130.

Fig. 25. Mandible (15-day). Decreased phosphatase activity of the definitive osteoblasts centrally. × 130.

Fig. 26. Ribs (16-day). Note phosphatase activity of the early periosteum and beginning activity of the hypertrophic chondrocytes. × 130.

Fig. 27. Periosteum of humerus (16-day). Note maximal enzyme activity in the pre-osteoblasts with decreased activity in the osteoblasts adjacent to the cartilaginous shaft below. × 540.

Fig. 28. Palatine suture (18-day). × 130.

Fig. 29. Radius (16-day). Cf. Pl. 2, fig. 13. × 100.

Fig. 30. Scapula (19-day). Note heavy enzyme activity in the hypertrophic cartilage and in the periosteum. × 100.

PLATE 4 (nucleoproteins and mitochondria)

Fig. 31. Part of scapula (16-day). Note the marked reduction in Feulgen-staining intensity of the definitive chondrocytes below as compared with that of the perichondrium above. × 130.

Fig. 32. Part of mandible (16-day). Feulgen stain. Staining is relatively heavy in the fibroblast and pre-osteoblast nuclei above but is much less in the osteoblast nuclei below. × 540.

Fig. 33. Mandible (16-day). Note conspicuous cytoplasmic basophilia of the centrally placed osteoblasts as compared with the more peripheral pre-osteoblasts. Pyronin and methyl green × 420.

Fig. 34. Tangential section through periosteum of mandible (18-day). Note conspicuous cytoplasmic basophilia of the osteoblasts adjacent to the newly formed bone matrix in the deepest zone of the periosteum. Methylene blue and eosin. × 130.

Fig. 35. Group of medullary osteoblasts from the mandible (20-day). Note the elongated mitochondria which avoid the vacuolar area of the cytoplasm. Author's silver method. × 1750.

Fig. 36. Periosteum of mandible (20-day). Note the numerous mitochondria of the osteoblasts below and their scarcity in the pre-osteoblasts and fibroblasts above. Author's silver method. × 605.

Fig. 37. An osteoclast from the mandible (20-day). Numerous rodlike and granular mitochondria. Author's silver method. × 1665.

Fig. 38. Periosteal osteoblasts from the mandible (20-day). The filamentous character of the mitochondria is clearly shown. Author's silver method. × 1665.

THE DEVELOPMENT *IN VITRO* OF THE ANTERIOR LOBE OF THE EMBRYONIC CHICK PITUITARY

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INTRODUCTION

Many embryonic organ rudiments when explanted and cultivated *in vitro* are capable of a remarkably normal histological and sometimes also anatomical differentiation (Fell, 1940 and 1951). This type of tissue culture, although a valuable tool in experimental embryology, has been rarely applied to the study of the embryonic development of endocrines.

Pituitary tissue from infantile mammals, when cultivated *in vitro*, has been shown to maintain its histological and possibly also some of its functional characteristics for a fairly long period (Gaillard, 1937*a*; Martinovitch, 1951). While it has thus been demonstrated that differentiated pituitary tissue can be maintained outside the body, no information is available on the cultivation *in vitro* of the embryonic organ.

It was thought that if the embryonic gland could be grown *in vitro* without serious deviation from its normal course of development, certain problems concerning the differentiation of pituitary tissue could be investigated. An attempt was therefore made to cultivate the pituitary rudiments of chick embryos and to study the behaviour of such isolates. The investigation was intended to serve as a basis for an experimental approach to the problem of the developmental relationship of the various cell types in this gland, which was broadly reviewed by Severinghaus (1938) from the histological standpoint.

MATERIAL AND METHODS

The eggs used were from either Rhode Island Red stock or a Rhode Island Red × Light Sussex cross.

Pituitary rudiments of 6-day embryos were cultivated. The gland was dissected aseptically as follows: the frontal part of the brain was lifted, the optic chiasma and the infundibular stalk were cut through and the whole brain was then removed, revealing the site of the pituitary on the floor of the skull. The dorsum sellae was already formed at this stage as an elastic bar of procartilage. By cutting through the dorsum and the basisphenoid procartilage, the gland was released from the surrounding tissues. The epithelial stalk and the enveloping connective tissue were then removed, the organ washed in Tyrode's solution, passed through embryo extract and explanted on the surface of the culture clot. The explants were transferred to fresh medium every 2 days. Cultures of pituitaries from embryos ranging from 4 to 13 days of age were also made. In all fifty-four pituitary cultures were grown.

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All the glands were cultivated by the watch-glass method (Fell & Robison, 1929), except the youngest rudiments (4 and 5 days of incubation), which grew better in hanging drop cultures.

For the first series of experiments the culture medium consisted of 6 drops of fowl plasma and 3 drops of embryo extract. At the beginning of the culture period the extract was made from 11-day embryos, but when the glands had reached the total age of 11 days (i.e. 6 days *in vivo*, 5 days *in vitro*) they were cultivated further on media made with extracts from progressively older embryos according to Gaillard's method of 'ascending range' (Gaillard, 1937*a*).

In the second series of experiments, fowl heart extract was substituted for embryo extract. It was made from the hearts of adult chickens by the method described by Margoliash (1947). The heart tissue was dehydrated with acetone, desiccated and ground into a powder which was extracted in the refrigerator for 24 hr. with 22 volumes of Tyrode's solution; the extract was then sterilized by Seitz filtration. In preparing the culture medium the heart extract was used in the same proportion as the embryo extract.

Camera lucida drawings of the cultures were made daily.

The explants were fixed in Zenker-formol solution after 1–24 days of cultivation. For comparison a series of normal pituitaries from embryos of 6–14 days of incubation was fixed in the same manner. All organs were cut at 4μ and stained with Heidenhain's azan modification of Mallory's connective tissue stain or Ehrlich's haematoxylin and Chromotrop 2R. The McManus-Hotchkiss periodic acid-leucofuchsin method was employed for both the normal and cultivated pituitaries.

NORMAL DEVELOPMENT

The normal development of the pituitary will be briefly outlined to provide a standard of comparison for the development of the gland in culture.

The anterior lobe of the pituitary arises as an invagination of the oral epithelium, known as Rathke's pocket, on the 3rd day of incubation (see Patten, 1950). On the 5th day the hypophyseal vesicle is formed by the partial constriction of Rathke's pocket from the buccal cavity (cf. Atwell, 1939).

The pituitary rudiment of the 6-day embryo (Pl. 1, fig. 1) consists of the two main components: the epithelial vesicle and the infundibular process. After being in temporary contact at the extreme tips, these two parts become separated by a layer of connective tissue at this stage. The walls of the hypophyseal vesicle consist of columnar pseudo-stratified epithelium, from which the first lobular processes are beginning to form. The hypophyseal cavity still communicates with that of the mouth by means of the hollow stalk.

During the 7th day of incubation elongated cords form from the walls of the hypophyseal vesicle. Many of the epithelial cells are in mitosis, and during the 8th and 9th day of incubation the gland grows very rapidly. The epithelial cords derived from the walls of the vesicle are long and bent, and are widely separated from each other by connective tissue (Pl. 1, fig. 3). At this stage the relative amount of mesenchymal tissue in the gland is maximal when compared with younger and older stages. On the 10th day of incubation the residual lumen is obliterated. The first signs of cell differentiation are evident and in the central part of the anterior lobe a few acidophil and basophil cells are seen.*

During the next 2 days of incubation the differentiation of acidophil cells spreads throughout the anterior lobe, but the basophils remain few (Pl. 1, fig. 5). By the 14th day all three characteristic cell types—acidophils, basophils, and chromophobes—are distinct. At this stage cell cords and lobules increase in number and displace the broad connective tissue strands (Pl. 2, fig. 7). After 16–18 days the dense glandular tissue has been formed and the histological differentiation of the organ is completed.

The formation of the neural lobe begins on the 9th day of incubation when the first lateral diverticula arise from the infundibular process (Rahn, 1939). During the next few days numerous evaginations are formed, giving the rudiment of the infundibulum a glandular appearance. The histological differentiation, however, takes place in the second half of the incubation period, during which the walls of the diverticula grow in thickness and glia cells and fibrils form.

DEVELOPMENT *IN VITRO*

(1) *Cultivation in plasma and embryo extract*

Observations on living cultures. A thin outgrowth of fibroblasts surrounded the explant by the end of the first day of cultivation. The tissue was opaque, only the red blood corpuscles, which remained in the small capillaries, being visible. On the 2nd day the outgrowth increased in area and density, spreading to a distance of about half the diameter of the explant. The explant enlarged somewhat and became transparent, the cell cords inside being visible under low magnification. On the 4th day the glandular part of the culture was surrounded by a comparatively thick capsule of connective tissue and became more sharply defined.

Almost all the cultures showed a moderate liquefaction of the medium from the 4th day. Three explants were situated on one side of the liquefaction pool, being only partly attached to the clot; scanty fibroblastic outgrowth spread through the pool, while a denser ring of fibroblasts extending from the explant surrounded the area of liquefaction. Such 'signet ring' formations were also observed in cultures of embryonic thyroid by Carpenter (1942) and by McCarrison & Sankaran (1933), and are considered by them as a sign of intense metabolism of the tissue in culture. The liquefaction was still more pronounced in cultures of pituitaries from younger, 4- and 5-day, embryos.

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* It should perhaps be pointed out that the traditional terms 'acidophil' and 'basophil' used to designate the chromophilic cells of the anterior lobe are misleading. When stained with basic dyes such as methylene blue, the pituitary cells display, in certain stages of their secretory cycle, marked cytoplasmic basophilia due to their ribonucleoprotein content (Desclin, 1940; Dempsey & Wislocki, 1945; Dempsey, 1948; Herlant, 1943). This was even more constantly observed in the acidophil and chromophobe cells than in the basophils. The secretory granules of the acidophils stain equally brightly both with acid dyes (azocarmine and acid fuchsin) and with basic dyes (safranin and basic fuchsin). The secretory product of the basophils, on the other hand, has a slight affinity for some basic dyes, e.g. methylene blue (Wolfe, 1949), but readily attracts aniline blue, which is an acid dye. Thus the terms 'α-cells' for the acidophils and 'β-cells' for the basophils seem preferable. However, in view of the common usage of the words 'acidophils' and 'basophils' they will be employed in this paper.

Upon each transfer of the culture to fresh medium many cells fell off the explant on to the clot and eventually degenerated. This shedding of cells was more marked in young cultures and could not be prevented even by very careful manipulation.

On further cultivation the tissue became more compact, but this did not affect its transparency. By the end of the culture period the pituitaries had become slightly smaller than when first explanted; the probable causes of this will be considered in the discussion.

Histological structure of the explants. In sections of a 6-day pituitary rudiment cultivated *in vitro* for 1 day, the tissue of the anterior lobe consists of epithelial cords two cells in diameter. The residual lumen is still present in the form of a narrow cavity situated slightly eccentrically (Pl. 1, fig. 2). Numerous mitotic figures are present. After 2 days' cultivation the residual lumen is obliterated and, except at the periphery of the explant where the spaces between the cell cords remain fairly wide, the cords are closer together, the connective tissue between them being reduced (Pl. 1, fig. 4).

By the 4th day of cultivation the tissue is compact, the cords being separated by very thin connective tissue sheets. From this stage the number of mitoses gradually decreases, although slight mitotic activity can be observed even after 14 days of cultivation.

In sections of cultures grown for 5–6 days the first chromophilic cells are seen (Pl. 1, fig. 6). The acidophil cells tend to form small groups while the basophils are single and scattered. The acidophilic granules, which are very distinct, develop in the cytoplasm at first on one side of the nucleus only. In the basophil cells the cytoplasm around the central nucleus at first stains blue, displaying the dust-like granules only after 2 more days of cultivation. The histological appearance of the tissue approximates to that of the pituitary of a 14- to 16-day embryo.

After 8 days' cultivation the three types of cells characteristic of the anterior lobe are differentiated. The acidophil cells acquire the normal elongated shape and are filled with orange stained granules, which shift the nucleus to one end of the cytoplasm (Pl. 2, fig. 12); several stages in the accumulation of the secretory granules in the cytoplasm can be distinguished. The basophils are globular in shape with a slightly eccentric nucleus; the cytoplasm has a flocculent appearance due to the presence of minute granules and small vacuoles (Pl. 2, fig. 11).

To stain selectively the scattered basophil cells the McManus-Hotchkiss periodic acid-leucofuchsin test was performed on sections of both cultivated and normal embryonic pituitaries. The glycogen present in the embryonic cells, which is also demonstrable by this method, was removed by digesting the sections with saliva prior to staining. After staining, in both explants and controls, a reactive granular material was found localized in cells usually stainable with aniline blue, thus revealing the mucopolysaccharide component of the secretory product of the basophils.

In all cultures the floor of the infundibular process was included and the neural tissue developed to a certain extent. The infundibular recess, single at the time of explantation, becomes infolded and subdivided into four to six diverticula. A thick layer of connective tissue intervenes between the neural and the anterior lobe tissues. The cells in the walls of the diverticula elongate and thin fibrils, probably of glia, are present after 6 days *in vitro*. No mitosis and no pyknotic nuclei are found in the cells of the neural tissue.

Although both the neural tissue and probably the lobi laterales were included in all cultures, in no case did a pars tuberalis develop. It is claimed by Gaillard (1937*b*) that not only the development, but also the maintenance of the pars intermedia and pars tuberalis depend on the intimate contact of these lobes with the neural tissue. In all the cultures of the present series, the neural tissue is surrounded by a thick layer of connective tissue.

A small proportion of degenerating cells showing pyknosis and karyolysis are present in the anterior lobe after 4 days' cultivation. In older cultures the simultaneous degeneration of many cells leads to the formation of cysts filled at first with obvious cell débris and eventually with blue staining material called 'colloid'. Being surrounded by healthy cells, the cysts resemble follicles. Similar cell degenerations are found in the normal developing pituitary and have also been observed in the sublingual and submandibular glands of mice both in the normal embryo and in cultures (Borghese, 1950). They probably represent the normal phenomenon of morphogenetic degeneration, the role of which in the development of epithelial organs was stressed by Glucksmann (1950).

After 24 days of cultivation necrosis begins in a small central area of the explant. In this area the cell-bodies are completely disintegrated and only karyolytic nuclei stand up darkly stained. The lobular arrangement of the tissue disappears progressively from the centre towards the periphery of the explants, as the connective tissue seems to degenerate first. At the periphery of the explants, however, apparently healthy cells are present, which stain an intense orange-red. Basophile cells were not found in sections of these cultures.

(2) *Cultivation in plasma and adult heart extract*

Observations on living cultures. In general, the pituitary rudiments grown on medium consisting of plasma and adult heart extract behaved in a similar way to those grown on plasma and embryo extract. On the 2nd day of cultivation the explants became transparent, the developing cords and lobules being visible. The fibroblastic outgrowth was as abundant as that on medium containing embryo extract and differed only in form, being of smaller area but of much greater density. This result confirms that of Margoliash (1947), who found that heart extract promotes the growth of fibroblasts *in vitro* as potently as embryo extract. After a few days' cultivation, a slight liquefaction of the clot took place in all cultures. When tissue was transferred to fresh medium, there was no shedding of cells as in the explants grown on plasma and embryo extract. At the end of the culture period the pituitaries grown on medium containing heart extract were slightly smaller, more compact and less transparent than those of the previous series.

Histological structure of the explant. In sections of the pituitaries grown on plasma and adult heart extract, the tissue is seen to have undergone its characteristic histological and cytological differentiation. The cell-cords are formed during the first few days of cultivation. After 6 days in culture the chromophilic cells appear for the first time, and after 2 more days numerous acidophil cells have differentiated (Pl. 2, fig. 10). These latter stain a bright orange-red, resembling the normal acidophils not only in their shape and structure but also in the staining reaction of their granules. As in the normal gland, they occupy the peripheral part of the lobules and cords (Pl. 2, fig. 8). The basophil cells, as in the embryo, are less numerous, but distinct and clearly stained. The tissue is very dense and the cell-cords are closely packed, being surrounded by extremely thin strands of connective tissue.

In comparison with the cultures on plasma and embryo extract the cytological differentiation of the pituitaries grown on culture medium with adult heart extract seems more pronounced. The chromophilic cells are more numerous, while the acidophil cells seem more densely filled with secretory granules and more deeply stained.

DISCUSSION

In general, the development in culture of the embryonic chick pituitary is remarkably like its normal development *in vivo*. Thus from the 7th to 10th day in the embryo and during the first 4 days *in vitro*, the most striking histogenetic change is the formation of cell-cords from the walls of the hypophyseal cavity. The cords are closer together and the amount of connective tissue between them is less in the explants, except at the periphery. The connective tissue capsule around the glandular part of the culture, being formed during the first few days *in vitro*, probably tends to prevent the loose distribution of the epithelial cords which characterizes the normal anterior lobe at this stage.

During the next 4 days, both in culture and in the embryo, cytological differentiation takes place in the anterior lobe. This process is somewhat retarded *in vitro*, as the first chromophilic cells appear in the explants at a total age of 11 or 12 days (i.e. 6 days *in ovo* + 5–6 days *in vitro*), whereas in the normal gland a few such cells are found on the 10th day of incubation. However, the further differentiation and distribution of the chromophilic cells in the anterior lobe are similar in culture and in the embryo. The puzzling behaviour of the basophil cells (Rahn, 1939), which are fairly abundant at about the middle of the incubation period but decrease in number during later development, can also be observed in culture. From these results it is clear that the formation of cords and the differentiation of the chromophilic cells take place almost normally *in vitro* and at about the normal rate.

As already mentioned, the older cultures are smaller than the original explants and very small as compared with the normal gland of corresponding total age. However, the actual volume of epithelial tissue at the end of the culture period is greater than at the beginning. During the first few days *in vitro* growth, as indicated by the presence of many mitotic figures, is very active and a comparatively large amount of connective tissue is present. The loss of connective tissue on each transfer probably accounts in part for the small size in culture of some epithelial organs, which *in vivo* embody also a certain amount of connective tissue (Fell, 1928; Carpenter, 1942). Unpublished data suggest that in the pituitary the presence of mesenchymal tissue is indispensable for the formation of new cords.

When compared with cultures of infantile mammalian pituitaries, the cultivated embryonic pituitary of the chick shows a striking difference in the behaviour of its chromophilic cells. The acidophil cells of young rabbit pituitaries lose their granules after 18 days in culture (Gaillard, 1937*a*), while the basophils are altogether absent in cultures of the rat pituitary (Martinovitch, 1951). In the embryonic chick pituitary the acidophil cells retain their granules even after 24 days of cultivation, when necrosis begins in the centre of the explant. The basophil cells differentiate in culture, but as *in vivo*, they decrease in number at a later stage.

According to Rahn (1939), the basophils of the embryonic pituitary are not secretory in nature, but are a transient form of embryonic chromophilic cells. This assumption is open to the following considerations. It has been shown that the ability of the secretory basophils of the adult pituitary to stain with aniline blue is associated with metachromasia with toluidin blue (Herlant, 1950) and a positive McManus-Hotchkiss reaction (Catchpole, 1949; Pearse, 1948). The last test is considered to be fairly characteristic of the mucopolysaccharide components of the secretory product of those cells. The positive McManus-Hotchkiss reaction in the embryonic basophils indicates therefore that their secretory product is similar to that of the adult basophils. The assumption that the embryonic basophils may be secretory, also receives indirect support from the fact that their appearance on the 10th day of incubation and their decrease in number in later development are respectively coincident with the appearance and diminution of the secretory principles in other endocrine glands, e.g. thyroids (Rudnick, 1932; Hopkins, 1935), adrenals (Sun, 1932) and islets of Langerhans (Potvin & Aron, 1927).

The almost normal differentiation of the embryonic pituitary tissue *in vitro* raises the question as to how far it is dependent on the presence of embryonic materials in the culture medium. The experiments with embryonic pituitary grown on a mixture of plasma and adult heart extract, however, show that the characteristic histological and cytological differentiation of the tissue takes place in culture medium derived entirely from the adult.

The results of this study provide a striking example of the typical development of an organ rudiment in tissue culture, and support the view that such rudiments have an inherent capacity to differentiate histiotypically in partial or even complete isolation from the normal embryonic environment. Moreover, they indicate that the organ tissue culture technique provides a valuable approach to the problem of the relationship between the various secretory cells in the anterior lobe and possibly also to other problems in the physiology of the gland.

SUMMARY

1. The pituitaries of 6-day chick embryos were cultivated *in vitro* on different culture media. One series was grown on a coagulum of plasma and embryo extract and another on plasma and adult fowl heart extract.

2. The explanted pituitaries continued to differentiate *in vitro* in an almost normal way in both culture media.

3. The formation of cell-cords and lobules was somewhat more rapid in culture than in the embryo.

4. After 6 days' cultivation typical basophil and acidophil cells differentiated in the anterior lobe tissue in all the explants.

5. The neural lobe developed to a certain extent as the infundibulum became subdivided into several diverticula and glia cells and fibrils differentiated in its floor.

6. The nature of the chromophilic cells of the anterior lobe and their differentiation are discussed.

We wish to express our gratitude to Dr H. B. Fell, Director of the Strangeways Research Laboratory, for placing the facilities of her laboratory at our disposal and for invaluable help and advice throughout the investigation. We are also indebted to Dr A. Glucksmann and Dr W. Jacobson for helpful suggestions. Our thanks are due to Mr V. C. Norfield and to Mr L. J. King for technical help and also to Mr G. Lenney for taking the photomicrographs.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Longitudinal section through a normal 6-day chick embryo pituitary. *inf.* infundibulum; *h.v.* hypophyseal vesicle. Ehrlich's haematoxylin, Chromotrop 2R. × 80.

Fig. 2. Section through a 6-day pituitary after 1 day's cultivation on plasma and embryo extract. Ehrlich's haematoxylin, Chromotrop 2R. × 160.

Fig. 3. Longitudinal section through a normal 8-day embryo pituitary, showing the epithelial cords separated by wide strands of connective tissue. Azan. × 1140.

Fig. 4. Section through a 6-day pituitary after 2 days' cultivation on plasma and embryo extract. The epithelial cords are packed closely together. Azan. × 1140.

Fig. 5. Longitudinal section through a normal 12-day embryo pituitary. The chromophilic cells appear black. (*a*) acidophil cell. Azan. × 1140.

Fig. 6. Section through a 6-day pituitary after 6 days' cultivation on plasma and embryo extract, showing acidophil (*a.*) and basophil cells (*b.*). Azan. × 1140.

PLATE 2

Fig. 7. Longitudinal section through a normal 14-day embryo pituitary, showing a lobule of acidophil and chromophobe cells. Azan. × 1140.

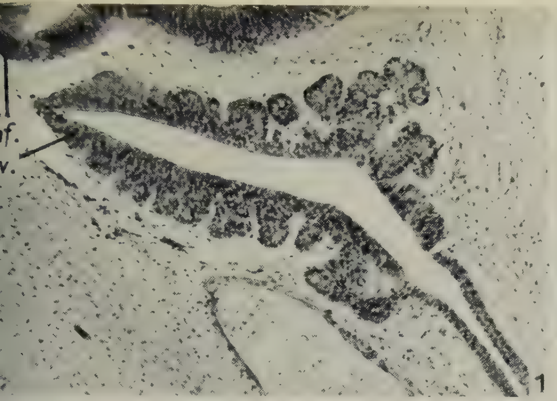
Fig. 8. Section through a 6-day pituitary after 8 days' cultivation on plasma and adult fowl heart extract. The acidophil cells are arranged peripherally in the lobules and cords. Azan. × 1140.

Fig. 9. Section through a 6-day pituitary after 8 days' cultivation on plasma and embryo extract. Azan. × 1140.

Fig. 10. Section through a 6-day pituitary after 8 days' cultivation on plasma and adult heart extract. The acidophils are larger and are more deeply stained than those in Fig. 9. Azan. × 1140.

Fig. 11. Different region of the same section as in Fig. 9. × 1710. *b.* basophil cell.

Fig. 12. Different region of the same section as in Fig. 9. × 1710. *a.* acidophil cell.



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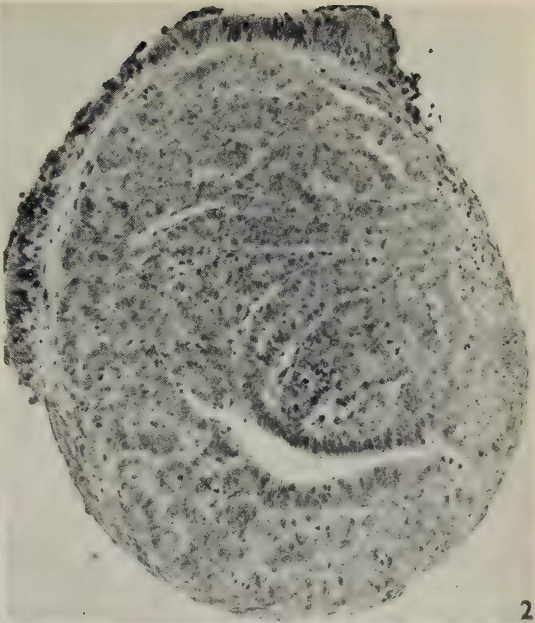
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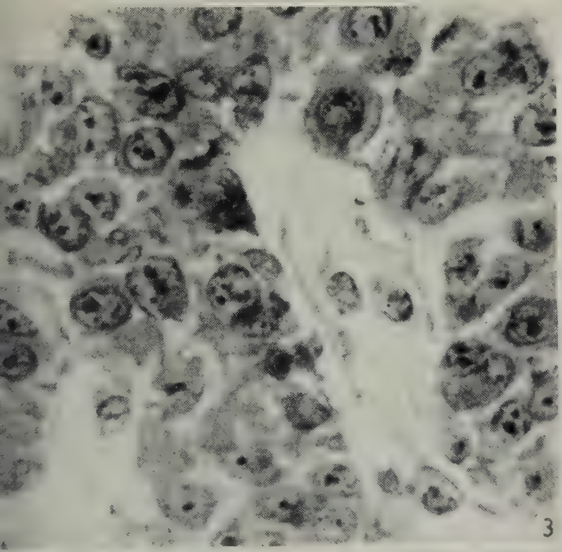
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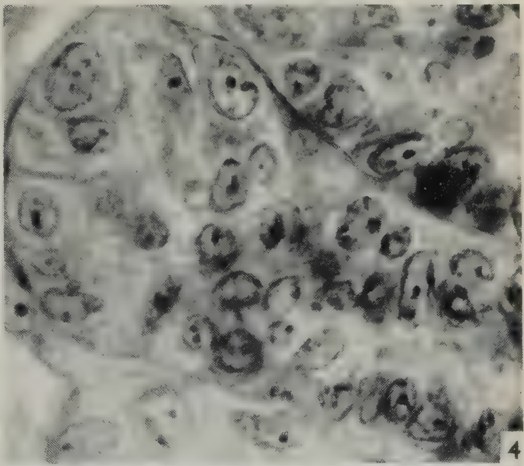
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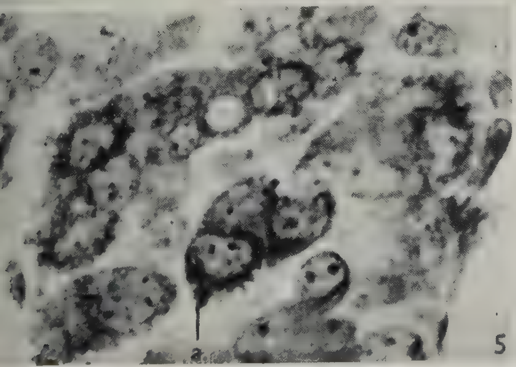

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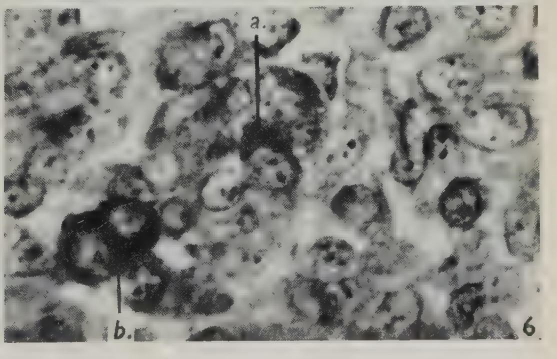

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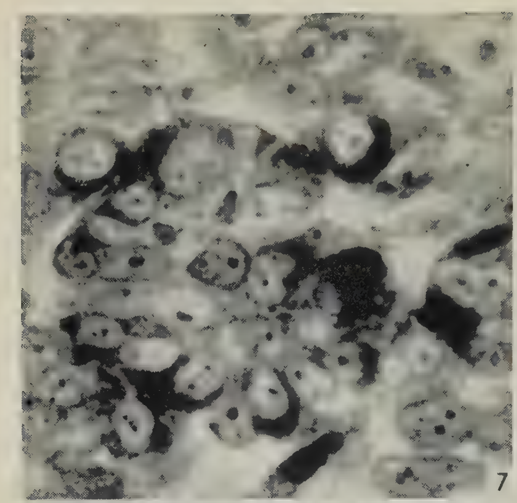
OSCONA and MOSCONA—Development of anterior lobe of embryonic chick pituitary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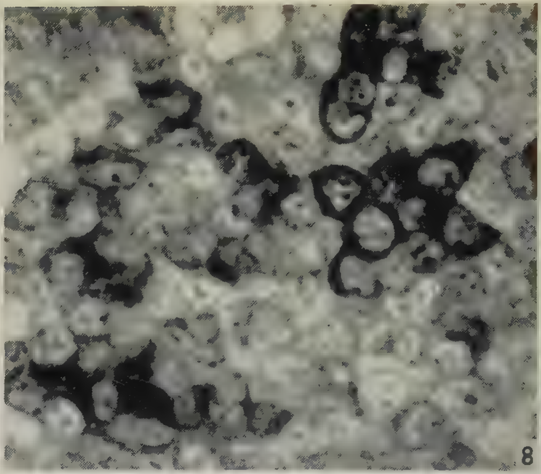
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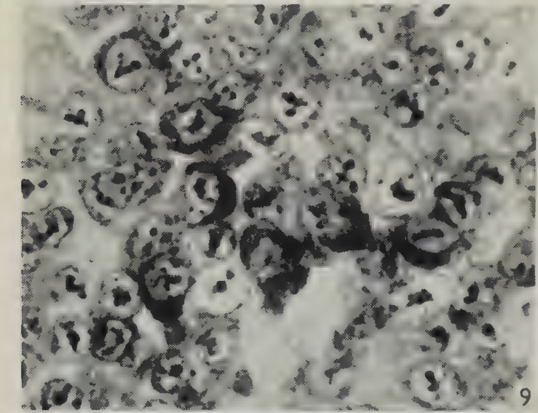
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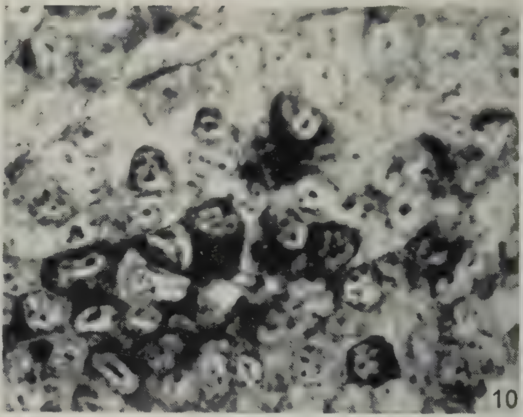
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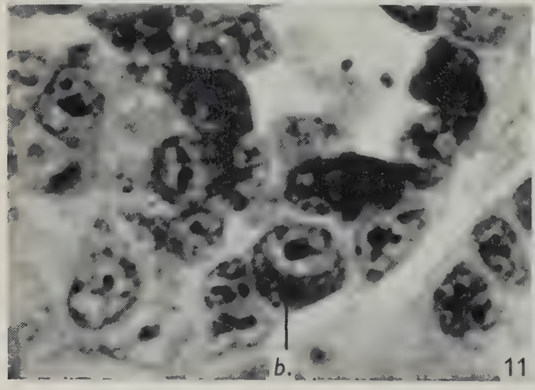

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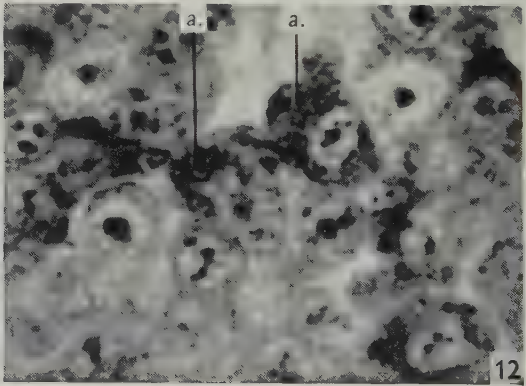

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MOSCONA AND MOSCONA—DEVELOPMENT OF ANTERIOR LOBE OF EMBRYONIC CHICK PITUITARY

THE DISSOCIATION AND AGGREGATION OF CELLS FROM ORGAN RUDIMENTS OF THE EARLY CHICK EMBRYO

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INTRODUCTION

The character and organization of tissues are determined by the spatial arrangement, the mutual relations and the typical groupings of cells which, together with the intercellular material, combine into developmental and functional patterns. To the structure and integrity of these cellular patterns are related the course of the prospective development of embryonic tissues and their characteristics when fully differentiated.

Many attempts have been made to study the capacity of these patterns for regulation and regeneration after their partial or complete destruction. It is well known, for instance, that in certain invertebrates, the tissues can be broken down into isolated cells which are able to reassociate into tissue-like structures; such reorganization of dissociated cells has been reported in sponges (Wilson, 1908; Galtsoff, 1925; and others), and in Coelenterates (DeMorgan & Drew, 1914; Chalkey, 1945). Furthermore, it has been shown that early embryonic amphibian cells also possess this capacity to a remarkable extent (Holtfreter, 1948*a*). In all these instances, however, the individual cells are either able to maintain themselves in isolation for some time as autonomous units (invertebrates), or they are provided with a sufficient amount of nutritive material, in the form of yolk, for a few days' survival in the isolated state (embryonic amphibian cells). With the tissues of warm-blooded animals, the experimental requirements are more exacting, the cells being absolutely dependent on adequate nutritional and thermal conditions.

In another communication (Moscona, A., 1952) it was shown that in the limb-rudiments and the mesonephros of early chick embryos, the cellular pattern could be disrupted by the progressive destruction of the intercellular materials by enzymatic digestion. The present study was undertaken to find whether the cells of these disrupted tissues could become reorganized into integrated systems and resume their presumptive histogenetic development.

By partially dissolving the intercellular material, the cells can be loosened and released from their mutual connexions so that their original spatial arrangement is greatly changed, while the continuity of the tissue is still maintained. Observations are reported on the behaviour of such partly disintegrated tissues when cultivated *in vitro*, as compared with the development of normal control explants.

If the intercellular matrix is completely destroyed the tissue can be dissociated into a suspension of discrete, viable cells which, under certain conditions of cultivation *in vitro*, reaggregate into clusters and so re-establish a tissue-like association.

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The formation and behaviour of these clusters have been studied in order to find how far the cells become reintegrated and whether they are able to acquire the characteristics of their tissues of origin.

MATERIAL AND METHODS

Material. The observations and experiments described were made on the leg-rudiments of 4-day chick embryos (40–42 somites) and on the mesonephroi of 3-day embryos (35–37 somites).

The dissociation of tissues. The procedure devised for the partial and complete disintegration of these tissues has been described elsewhere (Moscona, 1952), so only the main steps of the treatment will be mentioned here.

The organ rudiments were dissected aseptically and incubated for 15 min. at 38° C. in an isotonic saline lacking calcium and magnesium salts. They were then transferred to a 3% solution of commercial trypsin in this saline (sterilized by Seitz-filtration) at 38° C. After 10–12 min. in this solution, the epidermis of the limb-buds became loosened and could be easily peeled off. The loosening of the epidermis took place at the level of the basement membrane, so that a neat separation between the ectoderm and the mesoblast of the limb bud was possible, without the extensive loss of cells which usually accompanies dissection. Skinning with trypsin was also possible without pre-treatment of the limb-bud with the Ca and Mg free saline. As has frequently been reported (Medawar, 1941; Runnström, Monné & Broman, 1944; Northrop, 1947) trypsin, even in relatively high concentrations, does not damage living cells, and the present observations support this view.

The next step was carried out with fragments of the rudiments. The pH of the trypsin solution was gradually raised to 8·4–8·6 by addition of 1% KOH solution, and after 15–20 min. incubation the tissue collapsed into a shapeless mass of cells embedded in a viscous substance. If, at this stage, the medium was replaced by several changes of Tyrode solution, care being taken not to disturb the cells, after 8–10 min., the mass regained a certain degree of compactness. After thorough washing such partially disintegrated tissues were explanted for cultivation *in vitro.*

By prolonging the treatment with the trypsin medium at raised pH for a further 5–10 min., the cells became completely loosened and could be brought into suspension by squirting them once or twice through a pipette of a suitable bore. They were then washed twice by centrifugation in the Ca and Mg free saline and finally in Tyrode solution. At this stage they could be counted in a haemocytometer and the cell density in the suspension estimated.

All the operations with the tissues and the cells were performed, as far as possible, under microscopic control, and the timing and duration of the various stages of the treatment adjusted afresh for each experiment.

Tissue culture. Organ rudiments, tissue fragments and partially disintegrated tissues were cultivated by the watch-glass technique (Fell & Robison, 1929). The explants were grown on the surface of the clot and were transferred to a fresh medium every 48 hr.; grafting on the chorio-allantois of the chick was used in one experiment (see below).

In the case of the completely dissociated tissues, the discrete cells were resuspended in a fluid culture medium.* The cell concentration was usually adjusted to about 25×10^{-4} cells in 0·2 c.c. medium; 0·2 to 0·3 c.c. of the suspension was placed in the cavity of a hollow ground slide, sealed under a coverslip and incubated at 38° C. This type of preparation was also convenient for continuous observation of the cells on a constant temperature microscope stage.

Histology. The tissues were fixed in Zenker's solution with 4% glacial acetic acid for 1–3 hr. Sections were stained with Ehrlich's, Delafield's or Mayer's haematoxylin and counter-stained with eosin, chromotrop 2R or with Biebrich's scarlet. Sections were also stained with Heidenhain's azan and by the McManus-Hotchkiss periodic acid-leucofuchsin method.

Microscopical preparations of the cell suspensions were made by placing a drop of the suspension on a coverslip and fixing it by exposure for 10 min. to acetic acid-formol vapour. The cover-slip was then immersed face downwards in Zenker's fluid for 30 min. The cells were stained either with one of the haematoxylins or with Giemsa's stain.

I. EXPERIMENTS ON THE LEG-BUD

Histological structure of the 4-day leg-bud

The 4-day leg-bud is about 2 mm. in length. In section it is seen to consist mainly of diffusely arranged, stellate mesoblastic cells covered by a simple epidermis. The basement membrane appears at this stage as a thin lamella slightly thickened towards the apex of the limb bud.

In the mesoblast, an outer and an inner region merging imperceptibly into each other can be distinguished (Pl. 1, fig. 1). The inner region consists of a more condensed mass of cells which represents the future skeletal rudiment of the limb-bud and will be referred to as the *chondrogenic* or the *skeletogenic* blastema. Its cells are further distinguished by staining intensely with haematoxylin; at this stage they do not appear to be arranged in any orderly fashion.

The outer region consists of loosely bound mesenchyme cells and represents the *myogenic* blastema of the limb-bud. Minute blood vessels are concentrated in the area where the myogenic and the skeletogenic tissues merge.

The intercellular substance appears microscopically homogeneous. It gives a faint and diffuse McManus-Hotchkiss reaction which is more distinct in the chondrogenic region, where also some intracellular granules, resisting saliva digestion, can be demonstrated by this method; these may possibly represent the intracellular phase of the future mucopolysaccharide component of the extracellular matrix (Gersh & Catchpole, 1949).

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* The fluid culture medium consisted of the exudate from a fowl plasma-embryo extract clot. The exudate was collected after the clot had been crushed and incubated for 1 hr. at 38° C. (H. B. Fell, personal communication). This medium gave better results, in terms of reduced mortality of cells and more vigorous growth, than serum or unincubated exudate; this may have been due to the products of autodigestion released during the incubation (Fujii, 1941; Sanford, Earle & Likely, 1948).

The behaviour in vitro *of the partially disintegrated chondrogenic blastema of the limb-bud*

Object of experiments. To study the capacity of the isolated, partially disintegrated chondrogenic blastema to develop *in vitro* and to compare its behaviour in culture with that of a whole, skinned limb rudiment and the intact chondrogenic blastema.

Material and Methods. The development *in vitro* of the isolated chondrogenic blastema of the limb-bud has been described in detail by Fell & Robison (1929) and Fell & Canti (1934). Their descriptions served as a standard of comparison for results obtained in the following experiments.

Altogether, thirty-two 4-day limb-buds were used in the following four series of cultures. The first series consisted of five trypsin-skinned limb-rudiments explanted intact and cultivated for 4–5 days. Five similarly treated rudiments were explanted on the chorio-allantois of 8-day embryos and cultivated for 6 days; these formed the second series. The third series comprised five chondrogenic blastemata isolated by dissection from trypsin-skinned limb-buds and cultivated *in vitro* for 4–5 days.

The fourth series consisted of seventeen isolated chondrogenic blastemata partially disintegrated by treatment with the trypsin solution at raised pH. In this medium the tissue became very swollen due mainly to hydration of the intercellular material and partly to a slight swelling of the cells. The viscosity of the medium increased, presumably owing to the dissolved break-down products of the intercellular substances. The adhesiveness of the cells, both to glass and to each other, decreased; many of them rounded up and at the periphery of the fragment became detached. If in this condition the tissue was slightly prodded with a glass-rod, many cells fell off. When the trypsin medium was replaced with Tyrode solution, the intercellular material contracted, acquiring a sticky consistency and the whole fragment became gelatinous and dough-like. The fragments were then thoroughly washed with Tyrode; some were fixed while the remainder were placed for 2 min. in dilute embryo-extract and then explanted for 4–5 days' cultivation.

Results. The development *in vitro* of the trypsin-skinned limb-rudiments (first series) and of the isolated chondrogenic blastemata (third series) proceeded in essentially the same way as described by Fell & Canti (1934). In all these cultures the proximal skeletal parts developed, so that after 4 days' cultivation the rudiment of the femur, tibia and fibula were present. The distal tarso-metatarsal parts were usually represented by one or two masses of cartilage which in some of the cultures were small or fragmentary, probably owing to the destruction of some of the peripheral chondrogenic tissue when the blastema was dissected out. The absence of any typical anatomical differentiation in the tarso-metatarsal region in all of these skinned explants can be explained on the basis of Saunders's experiments (1948) which demonstrated that the presence of the ectodermal apical ridge is necessary for the differentiation of the distal skeletal parts of the avian limb.

No myogenic cells differentiated, in spite of the fact that in the first series of explants the myogenic tissue was intact. The myogenic cells were usually shed during the first 24 hr. of cultivation and were lost in subsequent transfers. These results did not seem to be directly due to the treatment with trypsin. As will be shown later, myoblasts can appear in trypsin-treated tissue *in vitro* and in chorio-allantoic grafts of the trypsin-skinned limb-buds, typically differentiated muscle developed in addition to the skeletal parts.

The above observations were intended to serve as standards of comparison for the explants of partially disintegrated blastemata. Sections of these blastemata, fixed immediately after treatment, showed that the structure of the tissue had undergone profound changes (Pl. 1, figs. 2, 3). It consisted of small groups of cells and of individual rounded cells embedded in a greatly swollen and microscopically structureless matrix. The intercellular spaces failed to stain with aniline blue or with Delafield's haematoxylin and eosin, but gave a general feeble reaction with the McManus-Hotchkiss procedure, which was clearest in the intercellular spaces of the small cell groups.

The appearance of this tissue strongly suggested that the treatment with the trypsin medium had reduced the blastema to a loosely connected mass of cells held together, in an apparently random arrangement, by residual components of the original intercellular material; it may safely be assumed that in this condition, the original pattern of the tissue was extensively disrupted.

During 4 days of cultivation *in vitro*, these explants developed into the typical proximal components of the limb skeleton, similar to those which formed in the explants of untreated chondrogenic blastemata (Pl. 1, fig. 4). In about ten cultures of blastemata which had lost relatively few cells during the process of partial disintegration, the skeletal rudiments which formed were of characteristic shape and size. In those explants which during the treatment had lost more than about one-third of their original cell mass, the skeletal parts showed various deficiencies and sometimes complete lack of distinct anatomical structure, the severity of the defects being closely related to the extent of cellular loss during the procedure.

Sections of cultures fixed after 1 day *in vitro* showed that the cells had already resumed their stellate shape and re-established mutual contact. The intercellular spaces had decreased to normal dimensions, as a result of which the explant contracted during the first day of cultivation. On the following day many mitoses were present and in some regions the tissue had assumed the appearance of pro-cartilage. On the third day, two or three foci of chondrification were evident. Some degenerating cells were also present at this stage, particularly in areas of cartilage formation; as such cells are also common during the development of cartilage in the embryo, they possibly represented a normal feature of chondrogenesis in the explants also (Jacobson, 1932; Jacobson & Fell, 1941; Glücksmann, 1951).

The MacManus-Hotchkiss procedure revealed at this stage the presence of intercellular mucopolysaccharide material, either in the form of minute granules or as thin fibrillar streaks. Intracellular fine granules were also present, some of which were removed by digestion with saliva, while others persisted. The foci of chondrification expanded into the shape of the future skeletal elements, so that on the fourth day of cultivation, the typical limb-bone rudiments were present.

Blastemata, which had lost many cells during the treatment before explantation, were seen in sections to have developed into chaotic masses of cartilage; the greater the loss of cells the more abnormal were the cartilaginous structures. Thus, while histotypical differentiation would proceed even in small fragments of the chondrogenic blastema in spite of partial disintegration, if more than about a third of its original mass was missing, the regulation and development of the skeletal parts were greatly disturbed.

Conclusions. (1) Trypsin-skinned 4-day limb-buds and chondrogenic blastemata isolated from trypsin-skinned limb-rudiments, develop *in vitro* and from the skeletal parts of the knee-joint region. (2) The partially disintegrated chondrogenic blastema is able to re-establish the integrity of its tissue *in vitro* and to differentiate in a similar way to the normal, untreated explants.

The behaviour of discrete limb-bud cells

Object of experiments. To determine the capacity of discrete limb-bud cells to aggregate and the extent to which such aggregates will differentiate *in vitro.*

Material and methods. Suspensions of limb-bud cells were prepared by the method described in the section on techniques. In some experiments the suspensions were made from either the skeletal or from the myogenic blastema only and thus consisted predominantly of one type of cells, while in others they were prepared from the whole limb-bud and so contained a mixture of chondrogenic and myogenic cells.

Shape and movement of isolated cells. When cells were released from the tissue into the alkaline trypsin medium, they reacted by various changes in shape and behaviour. Some of these changes probably represented the reaction of the cell to isolation, while others should be considered as a more specific response to the experimental medium.

From the surface of the freshly isolated cells (Pl. 1, figs. 5, 6; Pl. 2, figs. 8–10) blister-like processes of variable length and shape were protruded. These protrusions, which often terminated in a knob-like thickening, were in constant movement, the effect being similar to the 'bubbling' of dividing cells in tissue culture. Runnström & Monné (1945) have shown that when unfertilized sea-urchin eggs are exposed to lytic agents, their cortical layer bulges out into hyaline blisters and pseudopods; Holtfreter (1948*b*) demonstrated that isolated amphibian cells put out plasmatic protrusions when placed in solutions with a high concentration of hydroxyl ions, and suggested that the effect may be due to a general or localized increase in the solvation of the cell membrane. In general terms, this interpretation may also apply in the present case and the plasmatic protrusions of the discrete limb-bud cells may have been due to hydration of the cytoplasm resulting from the dispersive and lytic action of the hydroxyl ions and the trypsin solution on the cell surface. The tips of the various cytoplasmic protrusions stained deeply with haematoxylin (Pl. 2, figs. 10–12) and bluish with Giemsa's stain thus resembling, in this respect too, the protrusions of 'bubbling' dividing cells.

In this condition the cells were particularly susceptible to injury and, if they were not washed and transferred to the culture medium, they soon showed signs of damage. Myogenic cells were more susceptible than the chondrogenic cells, and usually a higher percentage underwent cytolysis and karyolysis (Pl. 2, fig. 11).

After transfer to the culture medium the movement of the protrusions slowed down; they were retracted and the cells rounded up. During the following 20–30 min. some of the cells became attached to the glass of the culture vessel, flattened and assumed the shape and the movement of fibroblasts. Others, while retaining the rounded form, began to move on the glass by pushing out amoeboid 'pseudopodia' of varied shapes, some being rounded and blunt (Pl. 2, fig. 12*a*) and others long and tapering. Some cells were observed to move by pushing out an elongated protoplasmic process the tip of which gradually acquired a knob-like form increasing progressively in size (Pl. 2, fig. 12*b*); the cytoplasm became constricted until the cell was in the shape of two spheres, one consisting entirely of cytoplasm, the other containing the nucleus, with a thin cytoplasmic connexion in between (Pl. 2, fig. 12*c*); the nucleus then moved into the cytoplasmic sphere and the process began again.

Myogenic cells usually acquired the fibroblastic form soon after transfer into the culture medium, and after 4–5 hr. of cultivation the rounded motile cells were in a minority. On the other hand, the chondrogenic cells persisted longer in the rounded state, and in pure cultures, fibroblastic elements were evident only after 10–12 hr. of cultivation.

Aggregation of chondrogenic cells. When grown in liquid culture medium, the discrete cells aggregated into clusters. The formation of these clusters began during the first hour of incubation, and after 3–4 hr. usually several minute clumps were found at the bottom of the culture vessel. Some consisted of a few cells only, while others were just visible to the naked eye. During the following 24 hr. several of the aggregates enlarged to between 0·2 and 0·5 mm.

The rate of formation of these clusters was largely influenced by the shape of the culture vessel, and they formed most rapidly at the bottom of a concavity; if the suspension was spread on a flat glass surface, the aggregation of the cells was greatly delayed, although small clusters were present after 10–12 hr. Aggregation did not take place if the cell suspension was spread on the surface of a flat plasma-embryo extract clot, the cells became quickly attached to the coagulum, and after 30–40 min. most of them had assumed the form of fibroblasts (Pl. 1, fig. 7).

In a 24 hr. culture of a cell suspension some rounded cells always remained discrete. Although they appeared normal, they had apparently lost the capacity of adhering to other cells. In several instances they were sucked off with a pipette, separated from excess fluid by centrifugation and transferred to fresh culture medium, in which they were heaped together with the aid of a glass needle. They did not form any stable clusters, however, and during the following day became abnormally hydrated and finally cytolized.

If a freshly prepared culture of a cell suspension was frequently agitated by squirting it through a fine pipette during the first 8 hr. of cultivation, almost no cell clusters were formed. Upon further undisturbed cultivation, only very small aggregates appeared and most of the cells remained solitary. These cells too, could not be maintained in a healthy state for more than 48 hr. after their initial isolation.

After 24 hr. the aggregates were usually transferred to a fresh liquid medium for 48 hr. and then transplanted to a clot in a watch glass culture.

Histological structure of aggregates of chondrogenic cells. After 8 hr., the aggregates in sections were seen to consist of polygonal cells, the cytoplasm of which stained readily with haematoxylin. No mitotic figures were present at this stage (Pl. 3, fig. 15).

Sections of clusters fixed after 48 hr. cultivation showed that the cells had differentiated into procartilage which, during the next 24 hr., developed into typical cartilage (Pl. 3, fig. 16). At this stage each aggregate consisted of a nodule of cartilage surrounded by a thin layer of fibroblasts. When cultivated further, the original nodule of cartilage enlarged and secondary foci of chondrification developed in the fibroblastic outgrowth in its vicinity, so that the culture was transformed into a colony of cartilaginous nodules (Pl. 3, fig. 17).

Aggregation of myogenic cells. While the general remarks concerning the discrete chondrogenic cells also apply to the suspended myogenic cells, the aggregates of the two cell types behaved differently.

Aggregates of myogenic cells were usually small, reaching a maximum diameter of 0·2 mm. When transferred either to fresh liquid culture medium or to a clot, they rapidly assumed the fibroblastic form and dispersed by migration so that they could not be maintained for more than 36 hr. In sections, these aggregates were seen to consist of an inner, loose, net-like scaffolding of stellate cells, surrounded by an outer layer of one or two flattened cells.

Aggregates formed in mixed suspensions of chondrogenic and myogenic cells. These experiments were arranged in two series. The first consisted of twelve cultures in which the proportion of myogenic to chondrogenic cells was the same as in the limb-bud, as each culture consisted of cells from the complete mesoblast of the limb-rudiment. All the clusters formed in this series of cultures developed a nodule of cartilage, surrounded by a fibroblastic sheath which was much thicker than in the cultures of purely chondrogenic aggregates; both types of tissue merged imperceptibly into each other (Pl. 3, fig. 18). Upon further cultivation, the cartilaginous core expanded while the fibroblastic sheath became gradually reduced in thickness until almost the whole aggregate consisted of cartilage. Myogenic cells did not differentiate in these cultures.

The second series of experiments comprised eight cultures, which were prepared by mixing the cells of one chondrogenic blastema with the myogenic cells from two limb-buds. The aggregates formed in six cultures of this series developed in essentially the same way as those of the first series. In two cultures, however, several larger aggregates were formed (0·4–0·5 mm.), and these were cultivated in liquid culture medium for a further 3 days. Sections of these aggregates showed an inner core of cartilage, in this case sharply demarcated from the surrounding layer (Pl. 3, fig. 19). In this outer layer, consisting mainly of densely packed, elongated cells, numerous large, multinucleated elements had developed. These cells were ribbon-shaped, their cytoplasm contained fine fibrillar structures, and their appearance indicated that they were myoblasts (Pl. 3, fig. 20).

The presence of myoblasts in the outer sheath of these mixed aggregates suggested that, during either aggregation or the early development of the clusters of mixed cells, the two types must have become segregated or sorted out, so that the chondrogenic elements were situated inside the aggregate, while the myogenic cells formed the outer sheath.

Conclusions. (1) Discrete limb-bud cells aggregate and develop histiotypically. (2) Aggregates of chondrogenic cells differentiate into cartilage. (3) Aggregates of myogenic cells only are very unstable and disperse by outwandering. (4) In mixed aggregates, myogenic cells form the outer sheath of the cluster while cartilage develops in the interior; myoblasts appear rarely and only in cultures with a large excess of myogenic cells.

II. EXPERIMENTS ON THE MESONEPHROS

Histological structure of the 3-day mesonephros

The mesonephros of the 3-day chick embryo (Pl. 4, fig. 21) consists of tubules in various stages of development, arranged along the collecting duct. The anterior tubules contain a lumen continuous with that of the duct. In the posterior part of the mesonephros, some of the tubules have not yet acquired a cavity and form solid cell clusters. The mesonephros is embedded in a loose mesenchyme which closely envelopes the tubules and penetrates into the intertubular crevices.

Reaggregation of discrete mesonephric cells and the development in vitro *of the aggregates*

Object of experiments. To study the capacity of dissociated mesonephric cells to aggregate and develop into structures similar to their tissue of origin.

Material and methods. The tissues used in these experiments consisted of the tubules and duct of the mesonephros and the immediately surrounding mesenchyme, so that the suspensions prepared from this material were of mixed cells. In each set of experiments both the mesonephroi of the embryo were used and treated together. Cell suspensions were made and cultivated as described in the section on techniques.

Fourteen sets of cell suspension cultures were studied.

Results. Discrete mesonephric cells (Pl. 4, fig. 22) did not show the 'bubbling' reaction to the same extent as did the limb-bud cells. While still in the treatment medium they frequently formed blister-like protrusions and their cytoplasm became intensely vacuolated. Two main cell types could be distinguished: smaller cells, which tended to round up and contained numerous cytoplasmic vacuoles and relatively large cells which were usually elongated and protruded hyaline blisters from one end.

In the culture medium (Pl. 2, figs. 13, 14), the blisters usually subsided and both types of cells became rounded. After about 15–20 min. the cells began to move on the glass of the culture vessel by pushing out a hyaline pseudopodium. When two or more cells came in contact, they usually adhered, maintaining for some time their shape and movement and gliding along each other by means of their hyaline processes (Pl. 2, fig. 13). This movement gradually quietened down, the cells rounded up and became incorporated in a cluster.

The mesenchyme cells behaved essentially like the myogenic cells, but were not observed to form clusters.

After about 8–10 hr. of cultivation in a liquid medium, clusters of various sizes were present, the largest ranging from 0·1 to 0·3 mm. (Pl. 4, fig. 23). They lay at the bottom of the culture vessel and became linked together by the mesenchymal cells into short strings of bead-like clumps (Pl. 4, fig. 24). Some of these aggregates were maintained in liquid medium, which was changed daily throughout the culture period; others were transferred to a clot in a watch-glass culture. In certain respects, those in a liquid medium yielded more information on the potentialities of these cellular systems.

During cultivation the aggregates increased in size and transparency. Some of them elongated into cord-like structures with an inner translucent core. Usually secondary clumps appeared, so that small colonies consisting of primary aggregates and secondary formations were eventually established. The colonies were enmeshed in a loose outgrowth of fibroblasts.

In sections, the early aggregates appeared to be of two main types: some consisted of loosely packed cells without apparent orientation and without lumen; others formed hollow vesicles, consisting of one layer of columnar cells surrounding a cavity. No cell divisions were seen at this stage.

After 48 hr. nearly all the aggregates were vesicular. Many cell divisions were present. Further cultivation resulted in an increasing complexity of the vesicular and tubular structures (Pl. 4, fig. 25), some of which were elongated and built of columnar cells with finely granular cytoplasm, while others consisted of cuboidal epithelium. By staining with azan, different cell types could be distinguished, the identity of which, however, was not clear. In cultures maintained for 4–5 days, some of the vesicles contained a colloid-like homogeneous material probably of a secretory nature which stained with eosin or aniline blue (Pl. 4, fig. 26).

It could not be definitely ascertained whether all the mesonephric cell aggregates went through an initial solid stage before developing into vesicular structures, or whether some of the discrete cells became directly arranged into orientated epithelial vesicles. The impression gained was that both these types of development occurred. The normal 3-day mesonephros consists in part of tubules and in part of solid clusters, and it is possible that the primary pattern of arrangement of the reassociated discrete cells was related to the pattern of their original association in the intact mesonephros.

On the second day of cultivation, the colonies of aggregates became covered by a delicate capsule of elongated, flattened cells. This capsule was separated from the aggregates by a space, traversed by scattered fibroblasts. Care was taken to preserve the capsule intact during exchange of the liquid culture medium or during the transfer of the culture to a clot.

In cultures grown on a clot, the capsule usually thickened to form three to four layers of flattened cells, giving rise to an external outgrowth of fibroblasts (Pl. 4, fig. 26). In liquid medium, various remarkable changes took place in the structure of the capsule. In certain regions, the cells assumed a columnar shape, thus forming strips of columnar epithelium; elsewhere, the capsule became invaginated to form a hollow, finger-like process, projecting into the underlying space (Pl. 4, fig. 27*a*). Beginning at the inner end of such a process, the cells became transformed into columnar epithelium (Pl. 4, fig. 27*b*), the diameter of the lumen diminished and the whole structure developed into a tubule of columnar epithelium (Pl. 4, fig. 27*c*).

These formative changes took place at the surface when the differentiation of the primary aggregate was already well advanced. Thus, there appeared to be two waves of developmental processes in these cultures: first, the formation of aggregates by the discrete cells and their subsequent differentiation, followed some 24 hr. later by the development and invagination of the capsular cells and their organization into tubular structures.

Conclusion. Suspensions of discrete mesonephric cells form aggregates *in vitro* which differentiate into vesicular and tubular structures. The discrete cells are thus capable of re-establishing the structural pattern of their tissue of origin.

DISCUSSION

The results described above showed that the chondrogenic blastema was able to develop *in vitro* a nearly normal anatomical structure after the original cellular pattern had been extensively disrupted by the partial disintegration of the tissue. This pattern, therefore, must have become rearranged and the various presumptive regions re-established during the period of cultivation. That the avian limb-rudiment possesses a regulative capacity has been shown by experiments *in vitro* by Fell & Canti (1934) and in the living embryo *in ovo* by Rudnick (1946) and Saunders (1947). The results obtained by Fell & Canti suggested that there exists an inverse relationship between the degree of regulation and the amount of tissue removed: the present observations support this view, as it has been shown that regulation on the anatomical level would not take place if the partially disintegrated chondrogenic blastema had been reduced to less than two-thirds of its original volume.

In cultures of reaggregates formed in suspensions of discrete cells, reorganization of the cellular pattern proceeded to the level of histiotypical differentiation. Here, two main processes were involved: (1) the establishment of a cellular continuum from the discrete cells, i.e. aggregation; (2) the transformation of this primary association into an organized tissue pattern.

The fact that the cells aggregated most rapidly in concave vessels showed that gravitation accelerated this process. That active cellular movements might also play a part, however, was suggested by the observation that the cells aggregated, although slowly, on a flat surface. Since Roux (1894) first suggested the existence of 'cytotropic' forces of attraction between embryonic cells, various mechanisms have been proposed to interpret the process of cellular aggregation. Loeb (1920) advanced the idea that agglutination was the factor which made isolated cells join into tissue. In sponges, active amoeboid movement is mainly responsible for the aggregation of isolated cells (Galtsoff, 1925), while in Myxomycetes a chemical diffusible substance appears to influence the formation of plasmodia by the solitary cells (Bonner, 1947). Holtfreter (1948*a*), summarizing various aspects of the behaviour of isolated early embryonic amphibian cells, suggested that the meeting of such cells depended on chance, but that their subsequent cohesion to form clusters, or alternatively their mutual repulsion, was governed by certain physico-chemical conditions of their surfaces. His concepts of 'contiguity effects' and 'tissue affinities' (Holtfreter, 1939) may serve as a basis for interpreting the present observations on the stability or instability of clusters of aggregated myogenic cells and the 'sorting out' and reorganization of the cell types in mixed aggregates of limb-bud cells. Such phenomena as the formation of tubular mesonephric structures from solid aggregates and the polar orientation of cells, might also be explicable in terms of regional differences in the adhesiveness of cell surfaces. The inability of the discrete myogenic cells to form stable aggregates when isolated from the other constituents of the limb-bud, might also have been due to an impairment of their capacity to secrete *in vitro* the normal combination of the intercellular materials. If the chondrogenic cells were able to provide the missing substance it might explain why the myogenic cells cohered in the mixed suspensions of limb-bud cells.

Once a small cluster of cells is formed in the suspension, it may exert a directing influence on the discrete motile cells in its vicinity, by means of a fibrous colloidal exudate spreading from it on the substratum; as suggested by Weiss (1945), for nerve cell axon orientations *in vitro* fibres of this 'ground mat' may serve to trap migrating cells which come into contact with them and to orientate their course towards the cluster.

As would be expected, histogenesis in the cellular aggregates proceeded in accordance with the presumptive potencies of the tissues of origin. It should be emphasized, however, that the results and conclusions contained in this communication refer to tissues and cells whose developmental fate had already been determined, and no consideration has been given to conditions which may obtain in earlier stages, before the onset of final determination.

SUMMARY

1. A study was made of the ability of partially and completely disintegrated limb-buds of 4-day chick embryos and of completely disintegrated mesonephroi of 3-day embryos, to reintegrate, to re-establish their typical histological patterns and to differentiate further *in vitro*.

2. The chondrogenic blastema of the limb-bud, after partial disintegration by extensive destruction of the intercellular material, became reorganized and differentiated *in vitro* into the typical skeletal parts.

3. Discrete chondrogenic cells, obtained in suspension after complete disintegration of the chondrogenic blastema, reaggregated under suitable conditions of culture; the aggregates differentiated into normal cartilage.

4. Discrete myogenic cells from the myogenic blastema of the limb-bud, formed unstable aggregates *in vitro* which did not differentiate typically.

5. Aggregates formed in suspensions of mixed chondrogenic and myogenic cells consisted of an inner core of chondrogenic cells and an outer sheath of myogenic cells; myoblasts appeared rarely and only in mixed aggregates formed in suspensions with an excess of myogenic cells.

6. Discrete mesonephric cells in suspension formed aggregates, which differentiated *in vitro* into typical tubular structures. The formation of secondary tubules from a cellular capsule surrounding the primary aggregations, is described.

7. It is concluded that discrete limb-bud and mesonephric cells of the early chick embryo are able to re-establish *in vitro* a tissue-like association and to resume their characteristic histiotypical development.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Longitudinal section through a normal 2 mm. leg-bud of a 4-day embryo, showing the beginning of internal condensation to form the chondrogenic blastema. The square indicates the approximate boundaries of the excised blastema (fig. 2). Delafield's haematoxylin, eosin. × 38.

Fig. 2. Section through a chondrogenic blastema, partially disintegrated by treatment with the trypsin medium at pH 8·4. Azan. × 38.

Fig. 3. Part of the same section as that shown in Fig. 2. × 480.

Fig. 4. Section through a culture of a partially disintegrated chondrogenic blastema after 4 days' growth *in vitro*, showing differentiation of skeletal parts: *c*, femoral condyles; *t*, tibia; *f*, fibula; *tm*, tarso-metatarsus. Delafield's haematoxylin, eosin. × 38.

Fig. 5. Smear of a cell suspension of a completely disintegrated chondrogenic blastema. Ehrlich's haematoxylin, eosin. × 570.

Fig. 6. Smear of a cell suspension of a completely disintegrated myogenic blastema. Ehrlich's haematoxylin, eosin. × 480.

Fig. 7. Whole mount of a 24 hr. hanging drop culture of fibroblastic cells obtained from a suspension of limb-bud cells; the cells were grown on a plasma-embryo extract clot. Mayer's haemalum. × 480.

PLATE 2

Fig. 8. Smear of discrete chondrogenic cells from a suspension in the treatment medium. Ehrlich's haematoxylin, eosin. × 1140.

Fig. 9. Same preparation as in Fig. 1, cell in metaphase showing 'bubbling' cytoplasmic protrusions.

Fig. 10. Discrete chondrogenic cells immediately after resuspension in the culture fluid. The lower cell is in telophase. Ehrlich's haematoxylin, eosin. × 570.

Fig. 11 *a*, *b*. Smear of discrete myogenic cells from a suspension in the treatment medium, (*a*) cell showing elongated plasmatic protrusions; (*b*) fragmentation of the nucleus in a myogenic cell (top); cell with 'bubbling' processes (bottom). Ehrlich's haematoxylin, eosin. × 570.

Fig. 12 *a–c*. Locomotion of isolated chondrogenic cells in the liquid culture medium: (*a*) cell with blunt pseudopodium; (*b*) cell with elongated process formed by cytoplasmic streaming, leading frequently to formation of (*c*) cytoplasmic sphere connected by a thin stalk to the nuclear part of the cell. Ehrlich's haematoxylin, eosin. × 570.

Fig. 13. Mesonephric cells shortly after establishing contact, gliding along each other by means of protoplasmic processes. From a smear of a ½ hr. culture in liquid medium. Ehrlich's haematoxylin, eosin. × 950.

Fig. 14. Mesonephric cell in anaphase. Same preparation as in Fig. 13. × 1140.

PLATE 3

Fig. 15. Section through an aggregate formed in a suspension of chondrogenic cells after 24 hr. cultivation in liquid medium. *c*., small pieces of cotton-wool dragged by the aggregating cells into the cluster. Ehrlich's haematoxylin, eosin. × 240.

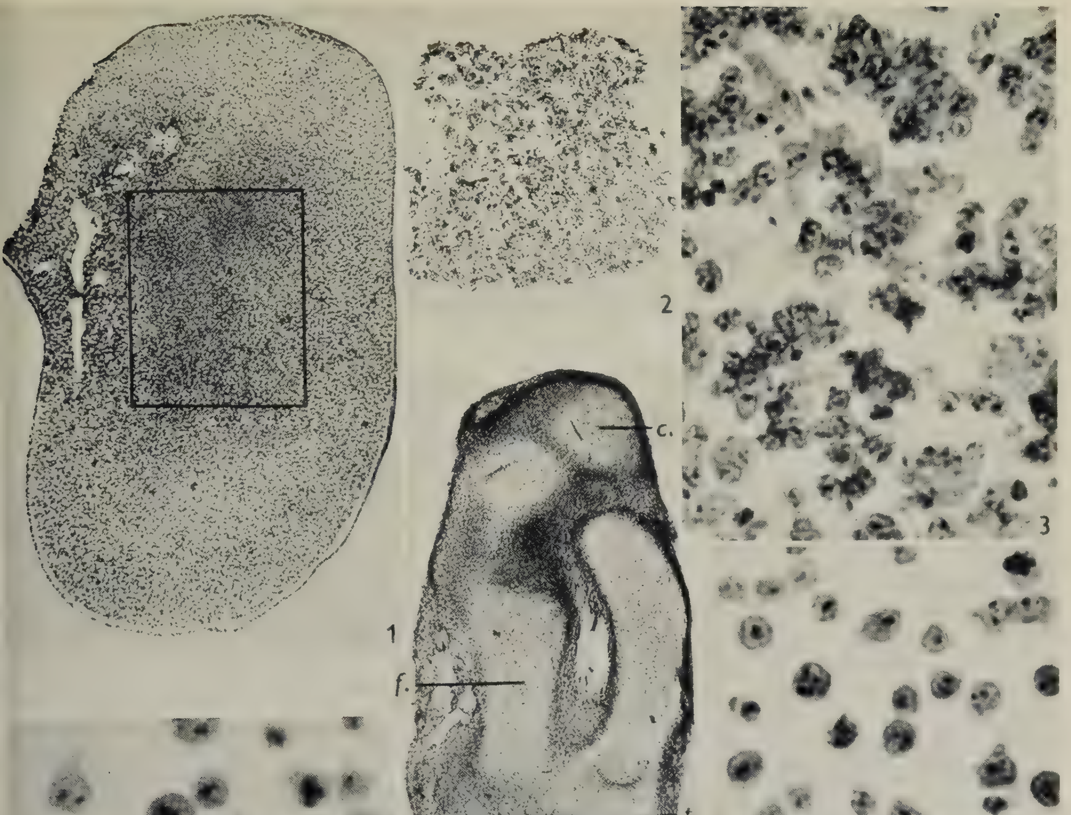
Fig. 16. Section through an aggregate formed in a suspension of chondrogenic cells and cultivated for 4 days. Note the well-developed cartilage. Ehrlich's haematoxylin, eosin. × 120.

Fig. 17. Section through a group of cartilage nodules formed in an aggregate of chondrogenic cells fixed after 8 days *in vitro*. Delafield's haematoxylin, eosin. × 60.

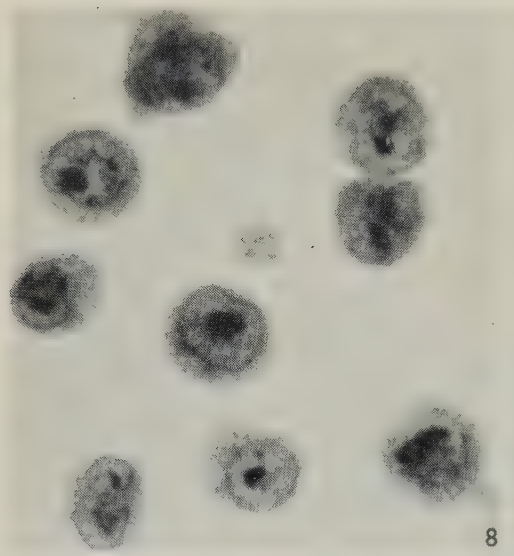
Fig. 18. Section through a 2-day culture of an aggregate formed in a mixed suspension of chondrogenic and myogenic cells. The inner part of the culture consists of chondroblasts (*ch.*) which merge imperceptibly into a more diffuse fibroblastic outgrowth of myogenic cells (*mg.*), McManus-Hotchkiss; Mayer's haemalum (20 sec.). × 240.

Fig. 19. Section through an aggregate formed in a mixed suspension of chondrogenic and myogenic cells in the approximate ratio of 1 : 2, fixed after 4 days *in vitro*. The internal nodule of cartilage is clearly demarcated from the surrounding capsule of myogenic cells. *c*., small pieces of cotton-wool dragged in by the aggregating cells. Ehrlich's haematoxylin, eosin. × 60.

Fig. 20. Part of the outer zone of the aggregate in fig. 19. × 480; note the multinucleated myoblasts. Ehrlich's haematoxylin, eosin.



MOSCONA AND MOSCONA—CELLS FROM ORGAN RUDIMENTS OF THE EARLY CHICK EMBRYO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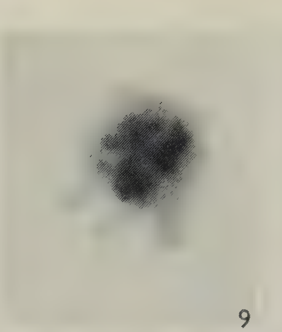
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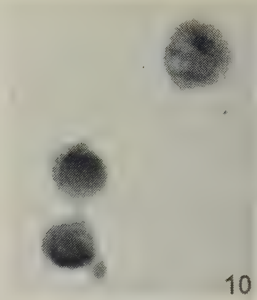
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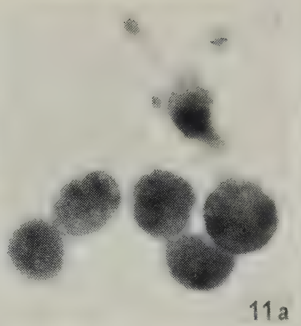
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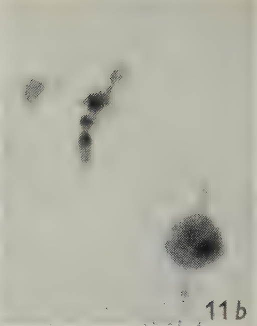
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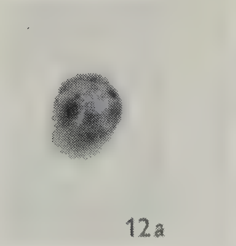
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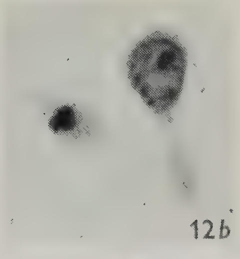
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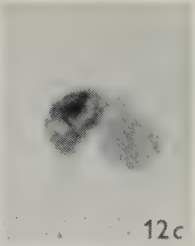
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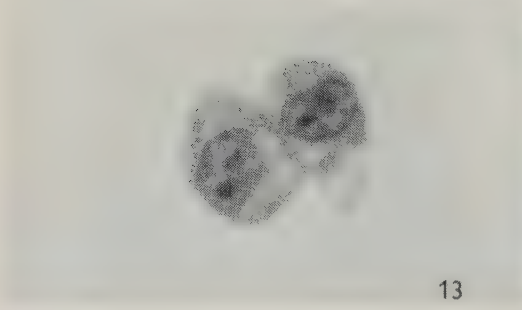
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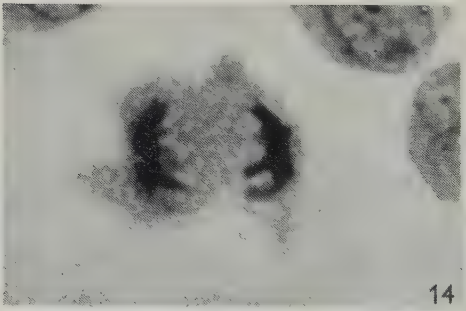
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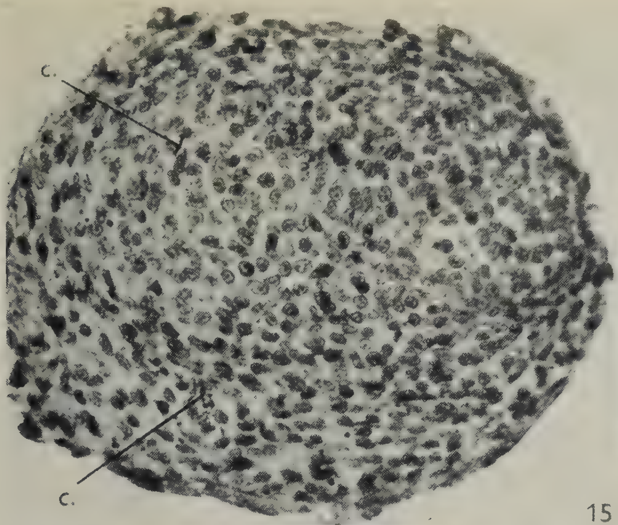
MOSCONA AND MOSCONA—CELLS FROM ORGAN RUDIMENTS OF THE EARLY CHICK EMBRYO



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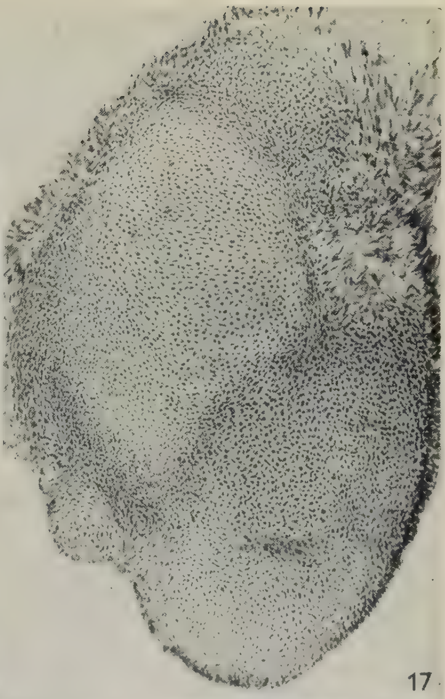

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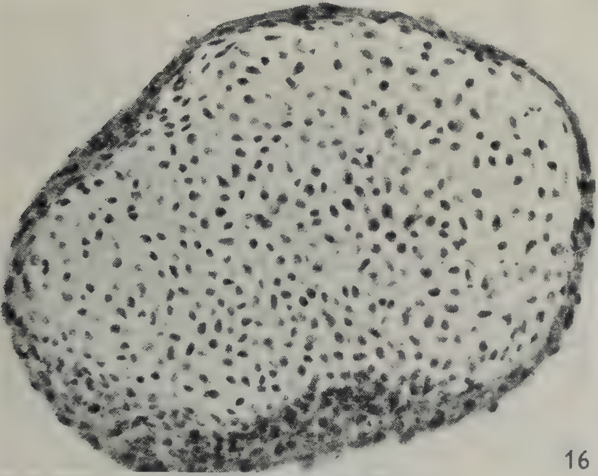

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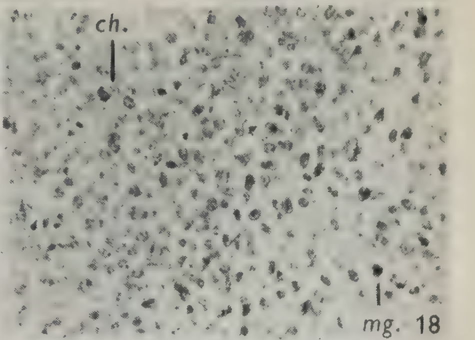

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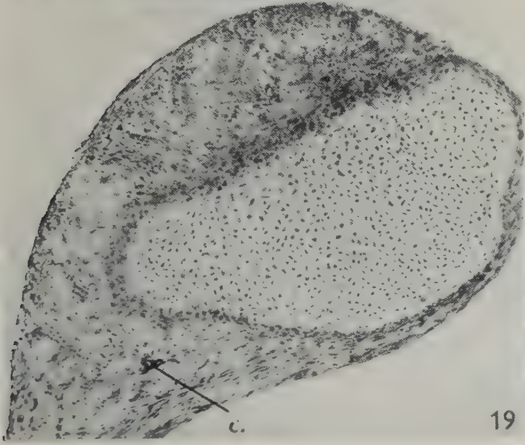

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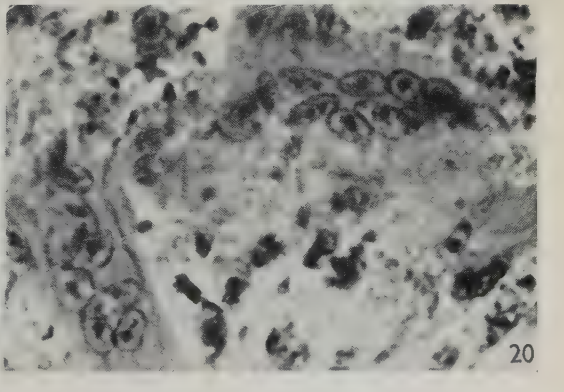

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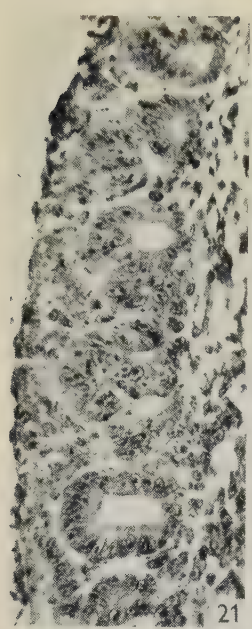
MOSCONA AND MOSCONA—CELLS FROM ORGAN RUDIMENTS OF THE EARLY CHICK EMBRYO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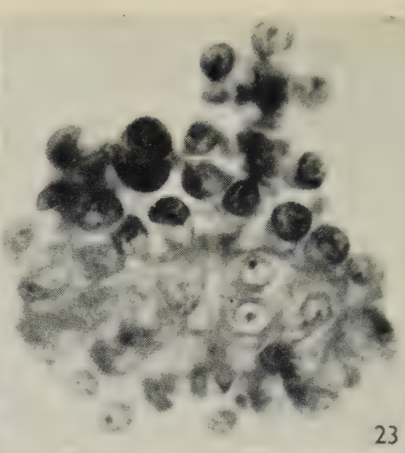
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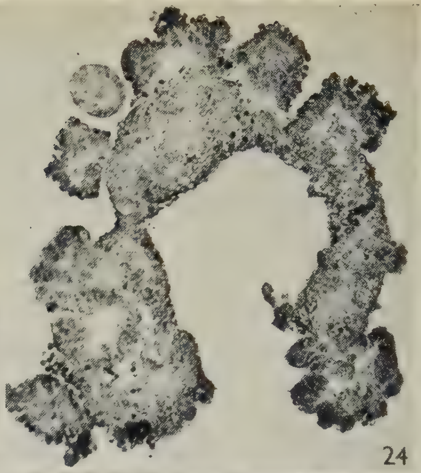
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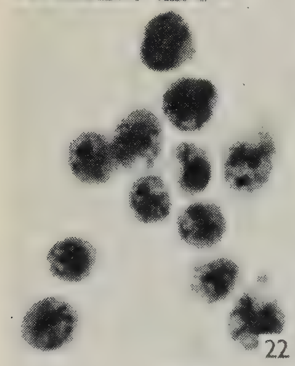
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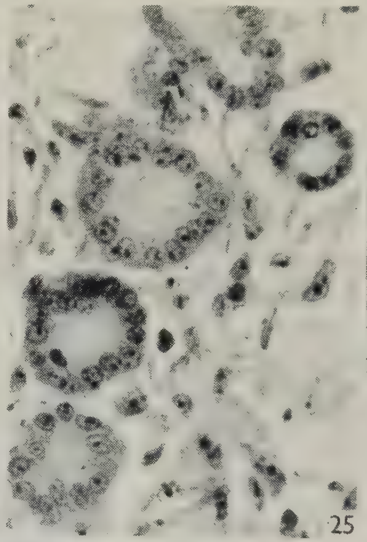
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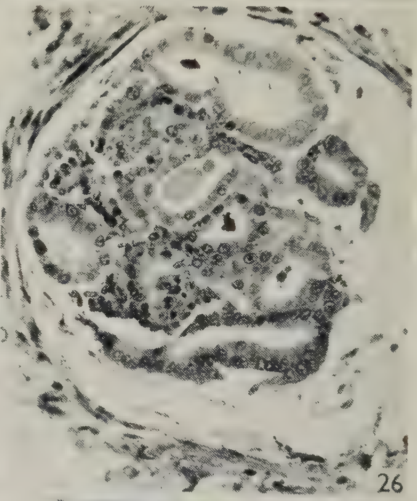
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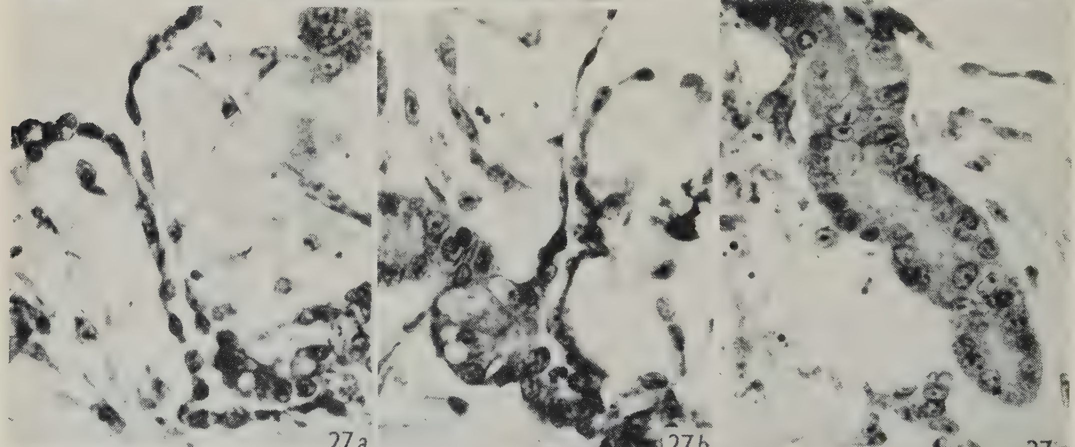
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MOSCONA AND MOSCONA—CELLS FROM ORGAN RUDIMENTS OF THE EARLY CHICK EMBRYO

PLATE 4

Fig. 21. Section through the anterior part of a normal 3-day mesonephros. Ehrlich's haematoxylin, eosin. × 240.

Fig. 22. Smear of cell suspension of a completely disintegrated mesonephros. Some cells show pseudopodial protrusions. Ehrlich's haematoxylin, eosin. × 570.

Fig. 23. Section through an aggregate formed in a suspension of mesonephric cells after 12 hr. cultivation in liquid medium. Ehrlich's haematoxylin, eosin. × 570.

Fig. 24. Section through a group of clusters formed in a suspension of mesonephric cells after 24 hr. cultivation in liquid medium. Clusters with and without a lumen are present. Ehrlich's haematoxylin, eosin. × 120.

Fig. 25. Section through a 3-day culture of aggregates of mesonephric cells showing differentiated tubular structures embedded in a network of fibroblasts. Ehrlich's haematoxylin, eosin. × 480.

Fig. 26. Section through a culture of mesonephric cell aggregates cultivated for 4 days on a clot. Tubular and vesicular structures are embedded in a fibroblastic network and surrounded by an outer capsule of flattened cells forming a radiating cellular outgrowth. A colloid-like substance is present in some vesicles. Ehrlich's haematoxylin, Biebrich's scarlet. × 240.

Fig. 27. Sections through mesonephric cell aggregates cultivated for 3–4 days in liquid medium. Secondary tubular structures are being invaginated from the single-layered capsule surrounding the culture. (*a*) Beginning of invagination into the fibroblastic stroma which occupies the space between the capsule and the primary aggregates; (*b*) appearance of columnar cells at the base of the invaginated process; (*c*) differentiation of columnar, uniformly orientated cells along the whole process. Ehrlich's haematoxylin, eosin. × 480.

THE CONDUCTION OF THE IMPULSE FOR CARDIAC CONTRACTION

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INTRODUCTION

Following the foundations laid by Engelmann (1875, 1878, 1894, 1895, 1897, 1900, 1903), Gaskell (1882, 1883, 1900), MacWilliam (1885), His, jun. (1893), Tawara (1906), Keith & Flack (1907) and Lewis (1916, 1921), it is widely believed that the automatic rhythmicity of the hearts of vertebrates is myogenic in origin, and that the impulse for contraction is transmitted throughout the several cardiac chambers, and from one chamber to the next, by the muscle fibres rather than by nerves. In mammals and birds, special muscular structures exist which are held to conduct the cardiac impulse, namely, the sinu-atrial node, the atrioventricular node and the atrioventricular bundle with its two limbs and their terminal branches (the ventricular subendocardial Purkinje fibres). Keith & Flack (1907) believed that these elements are remnants of more extensive tissues of similar structure in lower vertebrates. Davies & Francis (1941) found that the hearts of poikilothermal vertebrates (Fishes, Amphibia, Reptilia) contain none of these structures and that the musculatures of the several cardiac chambers have uniform histological characters. They therefore concluded that the special structures in mammals and birds are neomorphic in nature, undergoing parallel development in these vertebrates, and that, if the cardiac impulse in poikilothermal vertebrates is conducted by muscle, it is the ordinary myocardium that is responsible. The myogenic conduction of the impulse from atria to ventricles in mammals has recently been challenged, particularly by Glomset & Glomset (1940), Glomset & Birge (1945, 1948) and Field (1951 *a*), Glomset and his colleagues on the basis of their claim that in some mammals (dog, rhesus monkey and man) the atrioventricular (A.V.) node and bundle are either absent or feebly developed, and Field because in the A.V. bundle there are nerves which he suggested might be the agents that conduct the impulse. Many authors have noted the presence of nerves in the A.V. bundle of several mammals, while Cohn & Trendelenburg (1910) and Glomset & Birge (1948) stressed that in experimental lesions of the bundle, with resultant A.V. dissociation, the accompanying nerves are of necessity involved. Thus the experiments are not 'crucial' in determining which of the two elements of the bundle, muscle or nerve, conducts the impulse.

The present work involves a correlation between the time taken for the cardiac impulse to traverse the ventricles in mammals and birds and the degree of histological differentiation of the A.V. bundle and its branches, and it is submitted as collateral evidence in favour of the myogenic conduction of the impulse.

OBSERVATIONS

The *QRS* complex in electrocardiograms of mammals indicates the spread of the activation process throughout the ventricles; in birds, there is no preliminary *Q* deflexion in the electrocardiogram and the *RS* complex indicates this spread

(Lewis, 1916). Measurements were made of the duration of the *QRS* complex in several adult mammals and of the *RS* in two birds; they are expressed in hundredths of a second in Table 1. Some of the electrocardiograms were recorded by the present authors (sheep, cat, rabbit and wallaby). For some of the animals, measurements were made from published electrocardiographic records of other authors, e.g. cow (Alfredson & Sykes, 1942), man (Katz, 1941), dog, swan and pigeon (Lewis, 1916) and rat (Griffith & Jeffers, 1949). Electrocardiograms of the horse were obtained through the kindness of Prof. W. C. Miller, Equine Research Station, Newmarket.

Table 1. *The diameters of the muscle fibres of the atrioventricular bundle, the subendocardial Purkinje fibres and the ventricular myocardial fibres, the duration of the QRS complex in the electrocardiograms, and the heart rates* (*beats per minute*) *of a series of mammals and birds. The mammals and birds are respectively listed in order of decreasing sizes of the hearts*

Animal	A.V. bundle (μ)	Subendocardial Purkinje fibres (μ)	Ventricular myocardium (μ)	*QRS* (1/100 sec.)	Heart rate (calculated from e.c.g.)
Horse	35	88	12	7	44
Cow	30	40	12	9	71
Human	11	18	12	8	72
Wallaby	36	40	12	3·5	125
Sheep	30	40	12	3	180
Dog	12	18	12	4	180
Cat	9	15	12	4	240
Rabbit	10	14	12	3	187
Rat	9	13	12	2	300
Swan	35	44	9	3*	257
Pigeon	11	12	8	2·7*	182

* These figures refer to the *RS* complex, there being no *Q* deflexion in the bird (Lewis, 1916).

Measurements of the diameters of the muscle fibres of the A.V. bundle, the ventricular subendocardial Purkinje fibres and the ventricular myocardium were made on histological sections cut from adult hearts that had been uniformly treated by fixation in 10% formalin and impregnation in bulk by a silver-pyridine method (Blair & Davies, 1935). They were made directly, by means of a microscope eye-piece scale calibrated with a stage micrometer, on fibres which were cut longitudinally or transversely and through the central nuclei, excluding oblique sections and sections through the periphery of the fibres. Under these conditions the great majority of the fibres in each category were of the size stated in the table, though in each animal there was some range in the size of the fibres, the range being greater in the case of the Purkinje fibres than in the myocardial fibres. It was observed that the ordinary ventricular myocardial fibres in all the mammals examined showed a remarkable uniformity in diameter. On the other hand, the fibres of the A.V. bundle, as well as the subendocardial Purkinje fibres, show considerable differences in size from one mammal to another. In each mammal the subendocardial Purkinje fibres are larger than the muscle fibres of the A.V. bundle.

In the swan and pigeon, the ventricular myocardial fibres are smaller than those in the mammals, and the fibres of the A.V. bundle, as in the mammals, are smaller than the subendocardial Purkinje fibres.

DISCUSSION

Lewis (1921), under the title of 'The law of cardiac muscle', arranged the musculatures of different parts of the mammalian heart in sequence according to their properties as deduced from his experimental studies. For instance, in the dog both the diameter of the several fibres and the rate of conduction of the cardiac impulse in them were placed in the increasing order of A.V. node, ventricular muscle, atrial muscle, Purkinje fibres. This law applies to the different parts of one and the same heart, whereas the present work is a comparison between conditions in the hearts of different animal species.

From the results obtained, expressed in the table, the following comparisons may be made. First, among the mammals, the diameters of the muscle fibres in the A.V. bundle and of the ventricular subendocardial Purkinje fibres may be compared with the time taken for the cardiac impulse to traverse the ventricles (the *QRS* complex in the electrocardiogram). The *QRS* time (0·03 sec.) in the sheep is the same as that in the much smaller heart of the rabbit; the rate of conduction of the impulse is presumably, therefore, greater in the sheep. A notable difference between the two hearts is that the bundle fibres and Purkinje fibres in the sheep are about three times the size of those in the rabbit. The heart of the cow is much larger than that of the sheep; both the bundle fibres and the Purkinje fibres are respectively the same size in these animals, and the *QRS* time is three times as long in the cow (0·09 sec.) as in the sheep (0·03 sec.). The heart of the horse is larger than that of the cow, its bundle fibres and Purkinje fibres are larger, and the *QRS* time (0·07 sec.) is shorter than in the cow. The sizes of the bundle fibres and Purkinje fibres in general are related to the size of the heart, but that this is not necessarily so in all cases is indicated by a comparison of the human heart with that of the sheep. The human heart is much larger than that of the sheep, but the bundle fibres and Purkinje fibres of the sheep are much larger than in man; the *QRS* time in man (0·08 sec.) is much longer than that in the sheep (0·03 sec.). The *QRS* times in man, the cow and the horse are not markedly different, but the sizes of these hearts and the sizes of their bundle fibres and Purkinje fibres differ widely. The bundle fibres and Purkinje fibres in the cat are approximately the same size as those in the rabbit, but the *QRS* time is slightly longer in the cat (0·04 sec.), whose heart is bigger than that of the rabbit (*QRS*, 0·03 sec.). Likewise the bundle fibres and Purkinje fibres in man are about the same size as those in the dog, but the *QRS* time is much longer in the much bigger human heart.

The results expressed in Table 1 show that the duration of the *QRS* complex is not *solely* dependent on the size of the heart. For instance, the heart of the horse is very much larger than that of man, but the *QRS* time is actually slightly shorter, whereas the heart of man is larger than that of the dog and the *QRS* is also longer.

In all the mammalian hearts examined the ordinary ventricular myocardial fibres are the same diameter (12μ); it is only the fibres of the bundle and the Purkinje fibres which vary markedly in size. In each heart the subendocardial Purkinje fibres are larger than the bundle fibres; in man, cat, rabbit and rat the bundle fibres are actually smaller than the myocardial fibres and closely resemble them in histological structure. There thus appears to be a progressive differentiation, both as regards size and structure, of the fibres in the ventricular part of what is termed 'the conducting system' by those who hold the orthodox conception of the myogenic conduction of the cardiac impulse.

In general, it is seen that in hearts of unequal size, where the duration of the *QRS* is shorter in the larger heart, the fibres of the 'conducting system' are larger in the bigger heart (e.g. horse or cow compared with man; sheep compared with dog); where in hearts of different sizes the *QRS* is about the same, the bigger heart contains larger fibres in the 'conducting system' (e.g. dog and cat); and where in hearts of different sizes the fibres of the 'conducting system' are the same size, the *QRS* is longer in the bigger heart (e.g. cat and rat; cow and sheep). These comparisons in mammals suggest that there is a correlation between the diameter of the muscle fibres in the 'conducting system' and the rate of spread of the cardiac impulse through the ventricles.

This correlation also holds good in the case of the two birds examined. The heart of the mute swan (*Cygnus olor*) is very much larger than that of the pigeon; the fibres of its 'conducting system' are more than three times as large as those of the pigeon and its *RS* is only slightly longer.

The heart rates of the animals shown in Table 1 were calculated from the electrocardiograms; in each animal the rate is obviously subject to wide fluctuations. That in one and the same animal a varying heart rate does not influence the duration of the *QRS* complex is indicated, in man, by cases of sinus bradycardia and of tachycardia, in both of which the *QRS* is of normal duration. Electrocardiograms of horses at rest and after 20 min. severe exercise were loaned to the authors by Prof. Miller, and analysis of these shows that the durations of the *QRS* in the tracings of the hearts that had their rates accelerated by the exercise were the same as those of the resting animals.

Electrocardiographic records of a crocodile, *Crocodilus niloticus* (juvenile), were made by Davies, Francis & King (1951). The ventricles of this reptile have no A.V. bundle or Purkinje fibres, and the myocardial fibres are smaller (8μ) than those in the adult mammals examined in the present work. Although the heart of this animal is about the same size as that of an average-sized cat, the *QRS* complex in the electrocardiogram is much longer (0·13 sec.) than that in the cat (0·04 sec.), and indeed, longer than that in the mammals with much larger hearts. The slower rate of transmission in the crocodile, however, might in part be due to the lower body temperature (28° C.).

The above correlations are consistent with the orthodox view that in mammals and birds the cardiac impulse is conducted through the ventricles by the muscle fibres of the A.V. bundle and the peripheral Purkinje fibres, and they are submitted as evidence in support of that view.

Gasser & Grundfest (1936, 1939) have shown that the rate of conduction of an impulse in mammalian medullated nerve fibres is proportional to the diameter of the axis cylinders. In the present work a correlation has been made between the rate of spread of the cardiac impulse and the diameter of the fibres of the 'conducting system'. If these muscle elements are the true conductors of the impulse, the correlation may indicate that the phenomenon of conduction in them is a surface phenomenon as is known to be the case in nerves.

Finally, it is necessary to consider the nature of Purkinje fibres. The fibres originally described by Purkinje (1845) are the very large fibres seen, for instance, beneath the endocardium of the sheep or cow, and characterized by irregular distribution of cross-striated myofibrillae and of the multiple nuclei within the fibres (Blair & Davies, 1935). Such fibres are also present in the A.V. bundle of some animals, e.g. horse, cow, sheep, wallaby and swan in the series examined in the present work. But in some animals the fibres of the A.V. bundle are smaller than (man, cat, rabbit and rat), or about the same size as (dog) the ordinary ventricular myocardial fibres, and rather closely resemble the latter in histological structure. Also in the rabbit and rat the subendocardial 'Purkinje fibres' are only very slightly larger than, and differ only slightly in structure from, the myocardial fibres. Nevertheless, the bundle and peripheral fibres in all these animals appear to be homologous, and in the absence of any alternative name even the small peripheral fibres are generally referred to as 'Purkinje fibres'. The term 'neuromyocardium', as applied to the fibres of the bundle or the peripheral fibres, suggested in the Birmingham Revised Terminology (1933) is, in the opinion of the present authors, unsuitable and misleading as an anatomical term with structural implications.

Some authors have considered that Purkinje fibres are embryonic fibres of arrested development, though Field (1951*b*) maintained that this does not mean that the fibres are really embryonic but merely that they have been held up in development and failed to acquire their full complement of striated myofibrils. Many others, including the present authors (Davies & Francis, 1941, 1946), considered them to be specialized fibres on one or more of the following grounds. At no stage in its development does the ordinary myocardial fibre have the structure of a fully formed Purkinje fibre, so that the latter cannot be considered to be embryonic heart muscle or to be muscle arrested in its development. The Purkinje fibre develops from an undifferentiated cardiac mesenchymal cell and proceeds along its own line of development to become a fully mature Purkinje fibre, increasing in size and differentiating in structure, and becoming an entity which, it is believed, is capable of conducting an impulse at a faster rate than the myocardium, the latter becoming specialized in structure in association with its prime function of contraction. In cold-blooded vertebrates no such differentiation takes place, the musculature having uniform histological characters throughout the heart. It is in this sense that the present authors (Davies & Francis, 1941) suggested that the elements of the 'conducting system' of mammals and birds are neomorphic, undergoing parallel evolution in these animals and, as is generally believed, having the physiological property of a more rapid rate of conduction than ordinary cardiac muscle. Another observation in favour of considering Purkinje fibres (and nodal fibres) as specialized structures is the age changes seen in the sizes of the different muscle fibres in the marsupial heart (Davies & Francis, 1942). The fibres of the sinu-atrial and atrioventricular nodes attain their full size and structure in the foetus (pouch stage) of the wallaby, and are then considerably larger than the ordinary cardiac muscle fibres, the latter only attaining their full growth later. The fibres of the A.V. bundle in the foetus are already much larger than those of the myocardium; they subsequently continue to increase in size, and in the adult they are also much larger than the myocardial fibres even though the latter have also considerably increased in size. Similar changes have been noted in the dog by the present authors. There thus appears to be a differential rate of development between the fibres of the 'conducting system' and those of the ordinary myocardium.

Field (1951*b*) suggested that the changes in structure that Purkinje fibres in the sheep undergo during their development result from the relative inactivity of these fibres compared with the myocardial fibres, and that the changes are a differentiation (in its strict sense) rather than a specialization (implying a definite function). The present work, which indicates that a correlation can be made between the size of the fibres and the rate of spread of the cardiac impulse, is consistent with the view that the Purkinje fibres, varying in size and structure from animal to animal, are specializations related to the relative rate of conduction of the impulse.

The recent extensive experimental investigations in dogs by Sodi-Pallares, Rodriguez, Chait & Zuckermann (1951) showed that the spread of the impulse in the ventricular septum is in conformity with the distribution of the branches of the two limbs of the A.V. bundle. Davies, Francis & King (submitted for publication) found that the limbs of the bundle in the dog contain no nerves, and this observation, coupled with the work of Sodi-Pallares *et al.*, is submitted as strong evidence in favour of the conduction of the cardiac impulse by the bundle and its branches, rather than by nerves as other authors have suggested.

The results obtained in the present work suggest that it is possible to relate the time taken for the cardiac impulse to spread through the ventricles (*QRS* complex in the electrocardiogram) both to the degree of histological specialization attained by the muscle fibres of the 'conducting system' and to the size of the heart. In none of the animals examined was any exception found to this general thesis. The measurements of the muscle fibres in the hearts of the mammals and birds were not made from the same individuals as those from which the electrocardiographic records were taken; hence the correlations made above are expressed throughout only in general terms rather than as exact mathematical ratios.

SUMMARY

1. In a series of mammals and birds, the time taken for the cardiac impulse to traverse the ventricles, estimated from electrocardiograms, has been correlated both with the diameter of the muscle fibres of the A.V. bundle and of the ventricular subendocardial Purkinje fibres and with the sizes of the hearts.

2. The results are submitted as evidence in favour of the orthodox view that the impulse is conducted by the muscle fibres of the 'conducting system' of the heart.

3. Reasons are given for considering Purkinje fibres to be structures specialized for a particular function.

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THE EFFECT OF BACKREST POSITION ON THE PUSH WHICH CAN BE EXERTED ON AN ISOMETRIC FOOT-PEDAL

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THE PROBLEM

Mechanized agricultural operations may be divided into two broad classifications; those that should be carried out rapidly and those requiring great concentration at slow speeds. Both these tasks have become technically possible, but the limiting factor in mechanized farming may now prove to be the fatigue and discomfort experienced by the operator. This is mainly a question of the ease of manipulation of controls at all speeds and at all times during a prolonged operation.

While the development in design of tractors and new implements has proceeded rapidly the question of the seating position in relation to the controls of the tractor and its accompanying implement has been given little attention. Many variables must be studied before a final answer can be reached. Hugh-Jones (1947) has already determined the best position of a foot control relative to a seat, and his results are therefore taken as a starting-point for this investigation. The problem now is to find out, for a given position of seat and pedal, the height at which a backrest should be placed to enable the operator to exert a maximum push with the foot.

The problem is fundamentally one of stabilization. The assumption is made throughout that if the operator is working in the most advantageous position, then he will have a greater reserve of energy with which to combat fatigue.

THE APPARATUS

The apparatus is shown in Fig. 1. It consists essentially of a seat with an adjustable backrest, a pedal which can be placed in different positions relative to the seat and a rotating drum on which forces applied to the foot-pedal are recorded graphically.

The dimensions of the seat and backrest are those recommended by Weddell & Darcus (1947). The seat is 18 in. long and 15 in. broad, and is padded with a single layer of kapok approximately $\frac{1}{16}$ in. thick and covered with material to give a high coefficient of friction. The backrest, which is 5 in. deep, has a mean radius of curvature of $7\frac{5}{16}$ in. and is similarly padded and covered. It is held in position by two supports which slide in clamps so that any desired height of backrest can be obtained.

Two upright beams are bolted securely to the framework. These beams are drilled with bearing holes to take the supports on which the adjustable arms rest. By varying the height of these supports and the length of the adjustable arms the axle can be placed in any of a number of positions relative to the seat. The pedal and the recording arm are fixed to the axle.

The pedal, which is shown diagrammatically in Fig. 2, is $2\frac{1}{2}$ in. in depth and 5 in. wide, and is placed in the same plane as the subject's right hip joint. It is made to pivot about a stub axle and is constructed in such a way that pressure applied to the working surface is equivalent to a tensile pull at the hinge. Pressure on the back face, unless absolutely centrally applied, would cause the hinge to bend out of the straight, but pressure on the working surface causes the hinge to straighten automatically. This ensures that the pedal will not 'jack-knife' and a stable line of thrust is maintained through the pivot point for small pedal deflexions.

The recording arm is securely fastened to the axle at right angles to the pedal and is fitted with a spring-mounted Biro pen which is free to slide inside a copper tube.

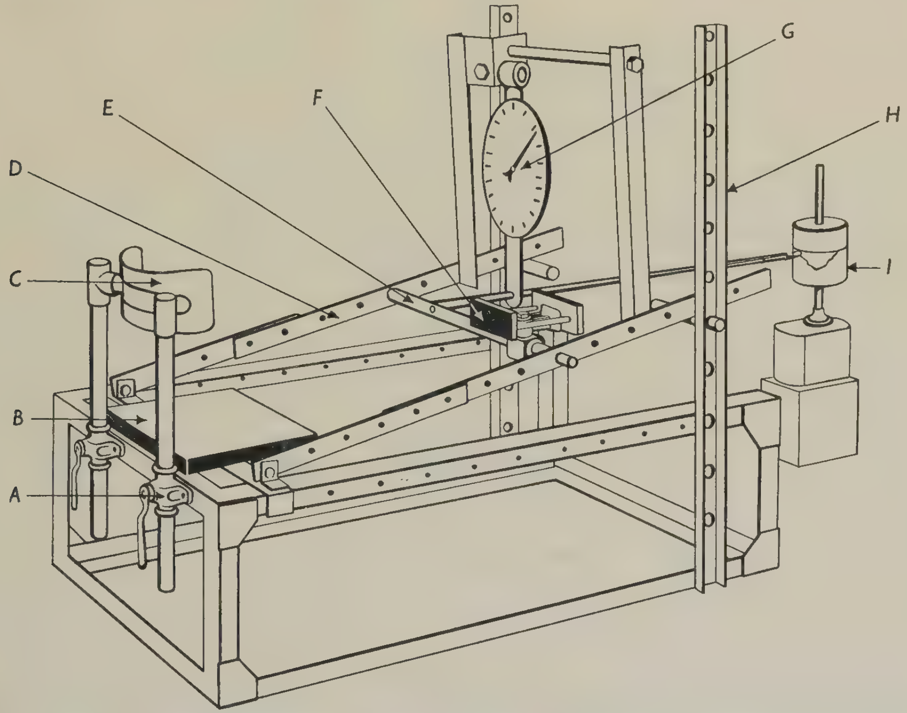

Fig. 1. Diagram of the apparatus. *A*, clamp; *B*, seat; *C*, backrest; *D*, adjustable arm; *E*, axle, *F*, pedal; *G*, spring balance; *H*, upright beam; *I*, recording drum.

As a thrust is applied to the pedal the pen traces a record on an electrically driven drum. A spring balance, which acts as load only, is also fitted to the recording arm. The subject is therefore able to see the result of his push.

Preliminary trials showed three things:

(*a*) It is undesirable to use a fixed bar as a pedal since the subjects complain that the bar causes pain when pushing against it. This means that the true maximum push is not recorded.

(*b*) A $5 \times 2\frac{1}{2}$ in. non-pivoting pedal does not give results which can be duplicated. It was found that the subject in exerting a large force tends to curl his toes inside his shoe. This transfers the line of action of the force from the centre of the pedal to its upper edge, increasing the moment of the force. This was done to varying extents and the results could not be reproduced.

(*c*) The subjects showed a preference to push with the ball of the foot (metatarsal heads) and so obtain a higher reading than with the instep. A chalk mark was therefore made on the subject's shoe opposite the ball of the foot. When pressure was applied this coincided with the horizontal line of the pedal.

It was also found that there was a great tendency for subjects either to hook the left knee around the seat and in this manner, by tensing the body muscles, obtain a higher reading for a maximum push, or to press their left foot on the ground. This generally resulted in a lower reading as energy was expended in lifting their own weight off the seat. The frame of the apparatus was therefore raised to such a height that all subjects' left legs could swing freely during the experiment.

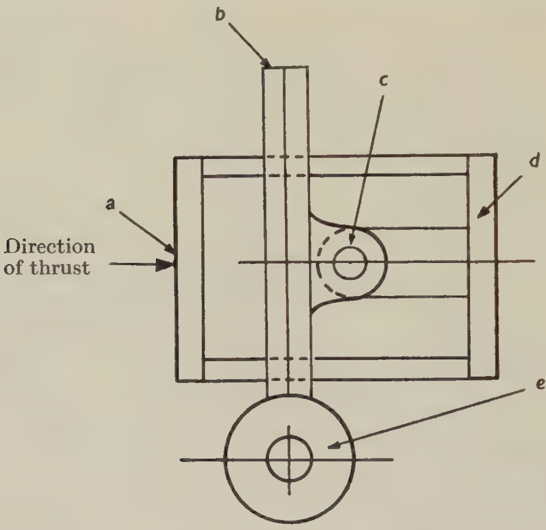

Fig. 2. Plan of the pedal. *a*, working surface; *b*, stub axle; *c*, hinge; *d*, back face; *e*, axle.

The apparatus was calibrated using weights up to 630 lb., and a graph was drawn relating the pen deflexions on the graphic record to the applied force. This graph was used to convert the forces exerted by the subjects during the experiments from units of pen deflexion into pounds.

EXPERIMENT I

To find how the maximum force that can be exerted on a pedal is related to the height of a backrest when the pedal is above seat level

Method

Ten male engineering students, between the ages of 20 and 30 years, were divided by height into two equal groups, those over 5 ft. $10\frac{1}{2}$ in. (the '*tall*' group), and those under 5 ft. $10\frac{1}{2}$ in. (the '*short*' group). Each subject in turn was seated on the apparatus with the back in an upright position. By suitable adjustments the pedal was placed in such a position that the angle between the subject's thigh and the horizontal was

15° and the knee angle, between the thigh and the calf, was 160°. This is the position which Hugh-Jones showed to be the best for obtaining a maximum push. The angles were measured with a template using the greater trochanter and the lateral epicondyle of the femur, the fibula head and the lateral malleolus as reference points.

The greatest force that could be exerted was found for five different positions of the backrest which were presented in a random order to each of the five subjects in a group according to a Latin square. All measurements of backrest height were taken from the seat to the centre of the backrest.

The positions were:

(*a*) No backrest.
(*b*) Centre of backrest 2½ in. from seat.
(*c*) Centre of backrest at waist level.
(*d*) Centre of backrest 13 in. from seat.
(*e*) Centre of backrest 17½ in. from seat.

For the purpose of these experiments the '*waist*' is defined as the position in which the subject feels the backrest fits his back most comfortably. The waist level was therefore determined subjectively, but it was found to be a well-defined position for each subject and, in general, coincided with the region of lumbar concavity, between the spines of the third and fourth lumbar vertebrae.

In order to obtain the feel of the apparatus, each subject was asked to press the pedal so that the reading on the spring balance was from 35 to 40 lb., to ease off, then press to obtain a reading from 70 to 80 lb., and again to ease off, then finally to exert his maximum. (This is a modification of a test procedure developed by Fisher & Birren (1945) for measuring hand strength.) The subject was then given a rest, the backrest was adjusted to a new position and the same procedure was followed to obtain another maximum. It was hoped that by this method fatigue and training effect would be small. All subjects had their arms folded so that as far as possible a standard position was adopted and the readings were not influenced by extraneous muscular contractions.

Each push produced a corresponding deflexion of the recording pen which was reproduced on paper fastened to an electrically driven drum. The subject was told to increase the force gradually and to hold his greatest push for a few seconds. A flat-topped curve was therefore produced. A 'ripple' was frequently seen on the graphic record as the force approached a maximum and the deflexion fell away slowly from fatigue if the position was held too long. Thus it was possible to see whether the subject was really pushing his hardest and sudden impulsive movements were prevented.

Results

Table 1 shows the maximum force in pounds that each subject was able to exert for every position of the backrest. The means and their standard errors are shown in Table 2.

The presence of a backrest, even in its lowest position, causes a very great increase in the force which can be exerted on the pedal. For both groups the push is greatest with the backrest at waist level. A second experiment was therefore designed to study this region more closely and to try to find the exact anatomical position in which the backrest gives greatest support.

Table 1. *The greatest force (in lb.) that can be exerted on the pedal by each subject with the backrest at different heights*

(Thigh 15° above horizontal, knee angle 160°.)

	Height of backrest above seat level (in.)				
	No backrest	2½	Waist level	13	17½
Tall subjects:					
1	50	300	327	335	232
2	45	285	492	455	268
3	111	263	530	484	443
4	72	277	595	521	422
5	45	183	255	285	262
Short subjects:					
6	28	243	300	300	238
7	100	213	320	277	263
8	60	195	372	313	350
9	45	232	320	277	264
10	88	353	460	455	335

Table 2. *The means and standard errors (in lb.) of the forces given in Table* 1

Height of backrest above seat level (in.)	Five tall subjects		Five short subjects		All ten subjects	
	Mean	±S.E.	Mean	±S.E.	Mean	±S.E.
No backrest	64·6	12·6	64·2	13·3	64·4	8·6
2½	261·6	20·5	247·2	27·7	254·4	16·4
Waist level	439·8	64·0	354·4	29·0	397·1	36·0
13	416·0	45·2	324·4	33·4	370·2	30·6
17½	325·4	44·3	290·0	22·1	307·7	24·0

EXPERIMENT II

To find how the maximum force that can be exerted on a pedal is related to the position of the backrest relative to the waist

Method

Twenty subjects were divided by height into equal groups, over and under 5 ft. 10½ in. They were weighed and the following measurements were taken with the subject standing upright.

(*a*) The distance between the greater trochanter and the lateral epicondyle of the femur. This was called the upper leg length.

(*b*) The distance between the head of the lateral condyle of the tibia and the under side of the sole of the shoe, opposite the ball of the foot (the plantar surface). This was called the lower leg length.

Each subject in turn was now made to sit upright in the seat, and the distance from the seat surface to the top of the iliac crest was measured. The backrest was moved up or down so that it attained the most comfortable position in the subject's estimation. This is the position that has been defined as waist level. It was found in nineteen cases out of twenty that the centre of the backrest in this position was at the same height ± ¼ in. as the top of the iliac crest.

For each individual subject this waist position (w) in. was marked on the backrest support. Four other positions of the backrest were also marked on the support, two above the waist and two below. The distance between each position was 2 in. The maximum push was then determined for these points ($w-4$) in., ($w-2$) in., (w) in., ($w+2$) in., and ($w+4$) in. The procedure for taking the readings was exactly the same as for Exp. I.

Results and conclusions

Table 3 shows the maximum force exerted by each subject for the different positions of the backrest. The means and their standard errors are given separately in Table 4.

Table 3. *The greatest force (in lb.) than can be exerted on the pedal by each subject with the backrest in different positions relative to the waist*

(Thigh 15° above horizontal, knee angle 160°.)

	Position of backrest relative to waist				
	Inches below waist		Waist level	Inches above waist	
	4	2		2	4
Tall subjects:					
1	375	389	413	418	414
2	382	422	393	488	506
3	418	443	442	470	422
4	356	378	340	300	295
5	288	320	310	318	350
6	423	455	490	455	500
7	277	320	372	404	378
8	305	327	335	320	263
9	225	250	282	335	313
10	213	242	260	317	310
Short subjects:					
11	318	407	389	404	350
12	250	375	360	432	385
13	253	313	335	303	310
14	363	435	448	448	404
15	318	335	343	368	407
16	295	347	317	357	347
17	347	327	397	425	335
18	244	238	250	247	267
19	353	388	418	421	401
20	295	305	363	442	324

Table 4. *The means and standard errors (in lb.) of the forces given in Table 3*

Height of backrest above or below waist (in.)	Ten tall subjects		Ten short subjects		All twenty subjects	
	Mean	±S.E.	Mean	±S.E.	Mean	±S.E.
4 below	326·2	23·9	303·6	13·9	314·9	19·4
2 below	354·6	23·8	347·0	18·0	350·8	14·5
Waist level	363·7	22·9	362·0	17·7	363·8	14·0
2 above	382·5	22·9	384·7	20·9	383·6	15·1
4 above	375·1	26·7	353·0	14·6	364·0	16·6

For both tall and short subjects the greatest push corresponds to a backrest position 2 in. above the waist. An analysis of variance shows that the differences between the forces exerted for the different positions of the backrest are significant at the 0·1 % level of confidence. Values of t were determined and these show that the $(w+2)$ in. position is significantly better than all the others while the $(w-4)$ in. position is significantly worse. Other differences may be attributed to chance.

It would be desirable when expressing the value $(w+2)$ in. as a height above the seat to know what is the mean height of the waist above seat level for a much larger sample of the population. Unfortunately none of the anthropometric data which are available gives this information. The results of the experiment can therefore only be interpreted in terms of the subjects who took part. The average height of the waist above seat level was 9·7 ± 0·8 in. for tall subjects and 9·0 ± 0·8 in. for short subjects. This means that the maximum force is exerted when the backrest is on an average 11·4 in. above the seat.

It was found that the maximum push exerted by an individual could not in this experiment be correlated with height, weight, lower leg length, upper leg length or total leg length. The mean force exerted by the tall group is greater than that of the short group for most backrest positions, but it will be noticed that the standard errors are also greater for the tall group. This suggests that some of these subjects are able to make use of their extra height to exert a greater force while others are not.

There is no doubt about the advantage to be gained by providing a backrest. It appears, moreover, from these experiments that the best position for the backrest is just above waist level, that is, approximately 11½ in. above the seat.

With the thigh inclined at an angle of 15° to the horizontal and a knee angle of 160°, it was found that in the majority of cases the pedal was 5 in. above seat level. While this position of a foot control is quite practicable in a number of cases, there are instances when the pedal must be placed below seat level. A third experiment was therefore undertaken to see if the same position of the backrest is best when the pedal is lower than the seat.

EXPERIMENT III

To find how the maximum force that can be exerted on a pedal is related to the height of the backrest when the pedal is lower than the seat

Method

Twenty subjects were divided equally into tall and short groups. For each in turn the pedal was placed in such a position that the thigh was horizontal and the knee angle 160°. The pedal was, on an average, 4½ in. below the seat. The maximum force that could be exerted on the pedal was measured for five positions of the backrest which were presented in a random order. The positions were such that the centre of the backrest was 6, 8, 10, 12 or 14 in. above the seat. In other respects the procedure was the same as in the previous experiments.

Results and conclusions

Table 5 shows the maximum force exerted by each subject for every position of the backrest. The means and standard errors are shown in Table 6.

The force which can be exerted increases as the height of the backrest is gradually raised from 6 to 12 in. There is little difference between the 12 and 14 in. positions.

An analysis of variance shows that the differences between the forces exerted at different backrest heights are significant ($P \leqslant 0{\cdot}001$). The t test shows that a difference of 35·75 lb. is significant at the 1 % level of confidence. This means that the 6 in. position is significantly worse than any of the others, and the 12 and 14 in. positions are significantly better. It follows that when the pedal is about 4½ in. below seat level, the backrest should be between 12 and 14 in. above the seat.

Table 5. *The maximum force (in lb.) exerted on the pedal by each subject with the backrest at different heights*

(Thigh horizontal, knee angle 160°.)

	Height of backrest above seat level (in.)				
	6	8	10	12	14
Tall subjects:					
1	300	235	350	364	385
2	238	285	277	277	320
3	183	220	238	326	364
4	326	448	422	483	435
5	277	350	408	422	435
6	321	292	225	263	225
7	159	183	183	213	220
8	177	195	188	170	147
9	195	255	270	305	270
10	170	220	244	292	364
Short subjects:					
11	220	255	326	364	335
12	165	177	220	225	277
13	213	225	225	270	335
14	200	292	244	350	335
15	213	225	270	343	380
16	195	300	364	305	270
17	147	207	225	263	244
18	277	292	335	350	300
19	220	270	350	428	407
20	255	385	385	371	335

Table 6. *The means and standard errors (in lb.) of the forces given in Table 5*

Height of backrest above seat level (in.)	Ten tall subjects		Ten short subjects		All twenty subjects	
	Mean	±S.E.	Mean	±S.E.	Mean	±S.E.
6	234·6	20·9	210·5	12·0	222·5	12·1
8	268·3	25·5	262·8	18·7	265·5	15·4
10	280·5	27·0	294·4	20·3	287·4	16·5
12	311·5	29·5	326·9	19·2	319·2	17·2
14	316·5	30·9	321·8	15·8	319·1	16·9

DISCUSSION

It is clear from the results of Exp. I that when no backrest is provided, only a comparatively small force of 64 lb. can be exerted on the pedal. This is, in effect, a measure of the frictional force between the subject's clothing and the seat surface. Even with the backrest in its lowest position there is an immediate increase of 200 lb., in the applied force. Under these conditions the subject no longer tends to push himself off the back of the seat. Slater (1949) has carried out a similar experiment using the typical ploughman's pan seat and he reported forces of only 177 lb. Here again, the subject was able to push himself over the edge of the seat.

Hugh-Jones (1947), on the other hand, used a backrest which supported the whole back and he obtained forces of the order of 850 lb. He admits, however, that his subjects were 'powerfully built and not a representative sample of the population'. The results of the present experiment are more comparable with those of Müller (1936) who found a maximum of the order of 440 lb. in a similar experiment.

Fig. 3 shows graphically the results of the three experiments which have been described in this paper.

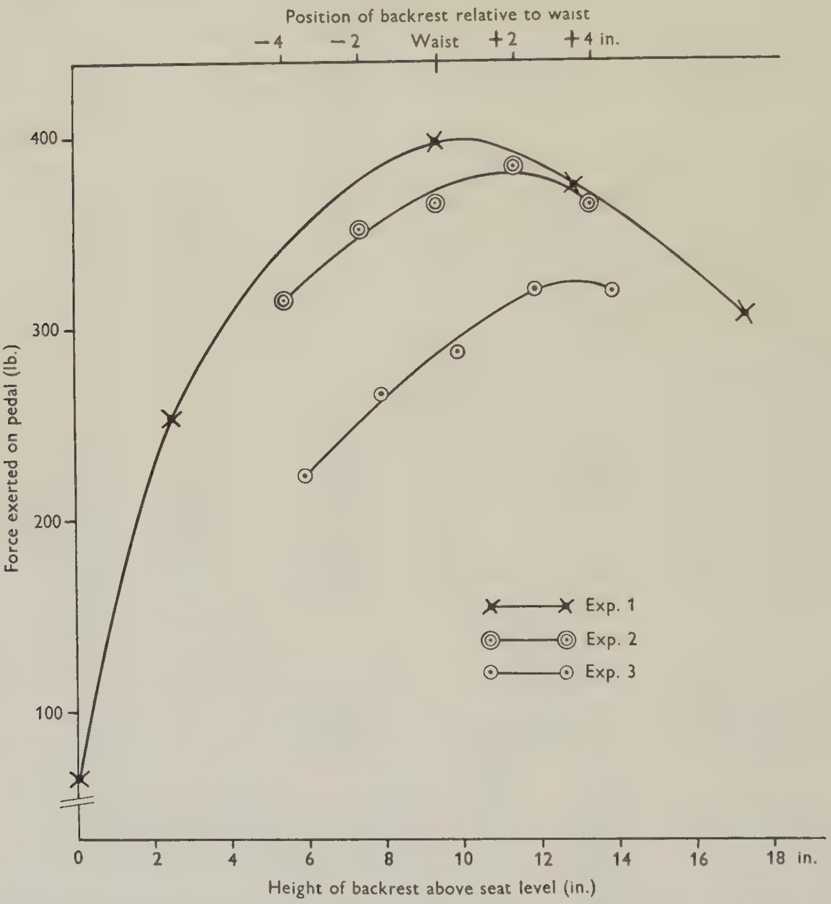

Fig. 3. Graph to show how the force that can be exerted on a foot-pedal varies with the position of a backrest. Exps. I and II, pedal above seat level; Exp. III, pedal below seat level.

A comparison of the results of Exps. II and III shows that as the pedal is lowered the optimum position of the backrest is raised. Müller (1936) and Weddell & Darcus (1947) both put forward a theory that for maximum push the backrest, knee and pedal should lie in a straight line. It follows from this that a lower pedal would require a higher backrest. If the straight-line theory is applied to the present experiment, backrest heights of 8·8 and 10 in. are predicted for pedals 5 in. above and $4\frac{1}{2}$ in. below seat level. The backrest positions which were shown to be best in the experiments are both about 3 in. higher than these theoretical values.

The graph also illustrates the fact that greater forces are exerted when the pedal is above seat level than when it is below. This might be attributed to individual differences between the subjects, but this is unlikely as twelve of the people taking part in Exp. III had also acted as subjects in Exp. II. The results agree with those of Hugh-Jones who studied various positions of an isometric foot-pedal relative to a seat and recommended that for maximum push the pedal should be between 7 and 8 in. above the seat to make the thigh angle optimum for 90 % of drivers.

SUMMARY

1. The problem is to find how the force that can be exerted on a pedal is related to the position of a backrest, 5 in. in depth. This is placed in different positions and the greatest force that can be exerted on the pedal is recorded.

2. When the thigh is 15° above the horizontal and the knee angle 160° the push is greatest with the centre of the backrest 2 in. above the waist, approximately 11½ in. from the seat. Under these conditions the pedal is about 5 in. above seat level.

3. When the thigh is horizontal and the knee angle 160° the push is greatest when the backrest is between 12 and 14 in. above the seat. In this case the pedal is about 4½ in. below seat level. The maximum force exerted under these conditions is not as great as when the pedal is in the higher position.

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GROWTH AND DIFFERENTIATION IN THE RED BLOOD CELLS OF THE CHICKEN EMBRYO

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INTRODUCTION

Thorell (1947*a*) has used measurements of haemoglobin to determine the degree of differentiation of developing red blood cells in adult mammalian bone marrow. The haemoglobin was measured in individual cells by the micro-spectro-photometric methods of Caspersson and his colleagues (see Caspersson, 1947) so that it was possible to determine the amount of haemoglobin in cells of different stages of development.

Dawson (1936) has shown that the circulating red blood cells in the chicken embryo, at any given time of incubation, are very largely at the same stage of development, their maturity increasing as incubation proceeds. By measuring the haemoglobin in suspensions of circulating red cells at different stages of incubation the haemoglobin content could be determined for cells at different stages of development. The suspensions contained insufficient developing white cells to affect the measurements. These have been compared with histochemical properties of the cells and with previously recorded measurements of average cell volume, number of dividing cells and the rate of respiration (O'Connor, 1951).

MATERIAL AND METHODS

Suspensions of the circulating blood cells of the chicken embryo were made and the number of dividing cells, the rate of respiration and the average cell volume were determined by methods previously described (O'Connor, 1951). To measure the haemoglobin content of the cells the portion of the suspensions used to determine cell volume was suspended in 2·0 c.c. normal saline and the number of cells checked. The haemoglobin was converted into alkaline haematin by the addition of 1·0 c.c. 2·5 % sodium hydroxide in normal saline and measured with a photo-electric colorimeter. The amount of haemoglobin per cell was thus determined and the concentration of haemoglobin (expressed as a weight/volume percentage) derived from the average cell volume. The earliest embryos on which haemoglobin determinations could be made were those at the end of 3 days' incubation when the developmental stage reached was that of 32–36 somites. Haemoglobin measurements were made from this stage until the end of incubation.

The general cytological appearances of the cells were studied in dried films made directly from the circulating blood and stained with Leishman's stain. The intracellular distribution of nucleic acid was studied by using Taft's (1951) mixture of methyl green-pyronin. A small drop of a suspension, or of blood taken directly from the embryo, was mixed with the stain on a slide, covered with a cover-slip, ringed with vaseline and examined as a wet preparation. Enzymatic controls were not used, for it was considered that the results of White (1947) justified the assumption that, in developing red cells, staining with pyronin indicated the presence of ribonucleic acid and staining with methyl green the presence of desoxyribose nucleic acid. Since this examination required only a small amount of blood it was possible to carry it out on the circulating cells of embryos of less than 3 completed days of incubation and at stages where the intra-embryonic circulation had just been established.

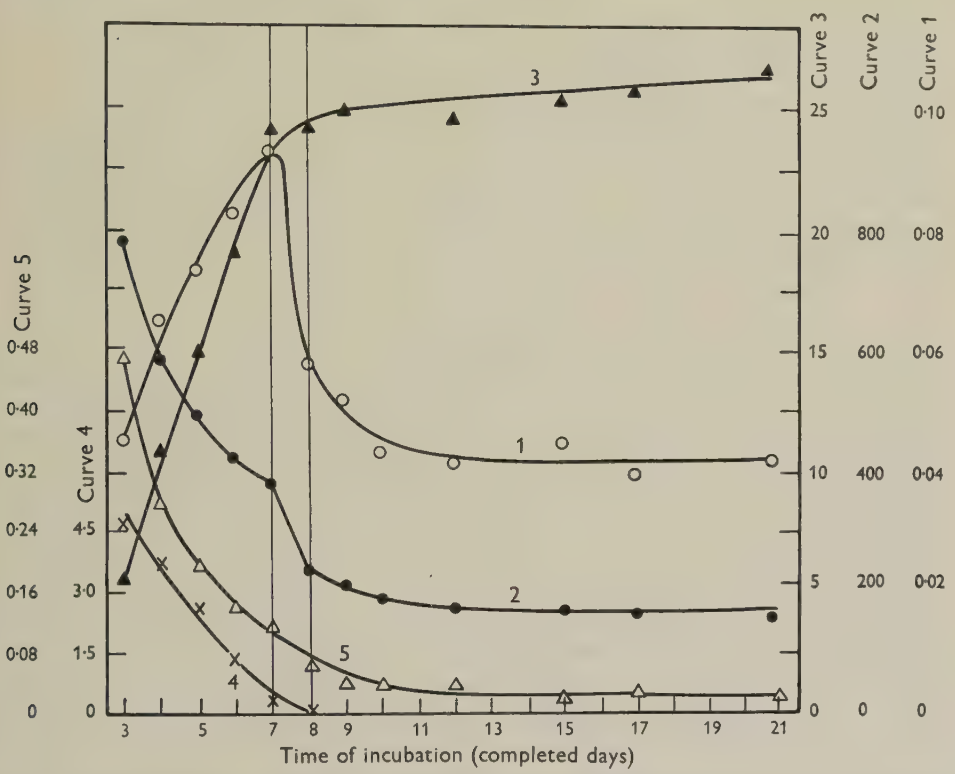

Fig. 1. The alterations in cellular characteristics of the circulating red blood cells of the chicken embryo during development. Curve 1, haemoglobin per cell ($\mu g. \times 10^{-3}$), O——O; curve 2, cell volume (μ^3), ●——●; curve 3, cellular concentration of haemoglobin (weight/volume: per cent), ▲——▲; curve 4, number of dividing cells (per cent), ×——×; curve 5, rate of respiration (mm.^{3} O_2/mm.^{3} cell substance/hr.), △——△.

RESULTS

Haemoglobin in developing red cells. For each completed day of incubation the average amount of haemoglobin per cell was calculated from eight to fifteen observations. These averages are recorded in Fig. 1, curve 1, which shows an increase until 7 completed days of incubation followed by a fall to reach, at the end of 10 days, a value which remains almost constant until hatching. Fig. 1, curve 2, records the previously determined values for cell volume from which the concentration of haemoglobin, recorded by means of curve 3, has been calculated. The haemoglobin concentration rises rapidly until the end of the eighth day and only slowly during the remainder of incubation. Previously determined values for the number of dividing cells and the rate of respiration are indicated in Fig. 1 by curves 4 and 5 respectively.*

Histological appearances. In the films stained with Leishman's stain the histological appearances and the predominant cell types at various times were essentially as described by Dawson (1936). In the wet films stained with methyl green-pyronin the cytoplasm of the cells circulating during the third day stained deeply with pyronin and there were one or more nucleoli with a similar staining reaction. These nucleoli were surrounded by material staining with methyl green ('nucleolus associated chromatin'). During the fourth day the pyronin reaction in the cytoplasm decreased, and the nucleoli disappeared leaving chromocentres stained with methyl green.† From the results recorded in Fig. 1 it was possible to determine that in such cells the haemoglobin concentration was 9–11 %. Nucleoli were not seen, except in an occasional cell, after this stage, although the cytoplasm stained with pyronin until the sixth day. Basophilia of cells stained with Leishman's stain persisted until about the same time. This staining reaction has been shown by Burt, Murray & Rossiter (1951) to be due to the presence of ribonucleic acid.

DISCUSSION

The variations in haemoglobin during development. It is apparent from Fig. 1 that between the seventh and tenth days there is a considerable fall in the amount of haemoglobin per cell with no corresponding decrease in haemoglobin concentration. Two possibilities can be considered as an explanation of this change. First, it might be due to cell division producing a diminished amount of haemoglobin in individual cells without a corresponding decrease in concentration. However, it will be seen from Fig. 1 that the decrease in the amount of haemoglobin in the red cells occurs when the number of dividing cells is zero or nearly so. A second possibility is therefore favoured. At any stage the characteristics of the circulating cells depend not only on the behaviour of the cells already in the circulation but also on the properties of those being discharged into the circulation from the sites of haemopoiesis. The observed changes in haemoglobin content would be accounted for if, at about the seventh day, the circulation received cells differing from those already circulating by containing a smaller amount of haemoglobin but a similar concentration because of a smaller size. Such an addition of smaller cells to the circulation is suggested by the changes of average cell volume indicated by curve 2 in Fig. 1. Until the end of the seventh day, and again after the eighth day, it is a smooth curve, but there is a sudden fall between the seventh and eighth corresponding to the fall in haemoglobin content of the cells. Two further points can be cited in favour of the change in haemoglobin values being due to the addition of a new population of cells to the circulation. First, Dawson (1936) has shown that after the sixth day the primitive blood cells become replaced by the definitive, and that this process is almost complete by the tenth day. Secondly, the sudden fall in the amount of haemoglobin in the developing red cells does not occur in red-cell formation in adult mammalian marrow (Thorell, 1947*a*) where there is no replacement of primitive cells by definitive. It is concluded, therefore, that the change discussed is due to the replacement in the circulation of primitive red blood cells by definitive.

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* Boyer (1950), using different methods, measured the respiration of red cells in chicken embryos of 14 days incubation and later. His values are considerably lower than those recorded here.

† In making this description the terminology of Caspersson (1950) is followed. The term nucleolus is confined to the pyronin staining material, and the associated material staining with methyl green is referred to as 'nucleolus associated chromatin' or, when the nucleolus has disappeared, as a chromocentre.

Growth and differentiation in red-cell development. Caspersson & Schultz (1940) have shown that the formation of protein by a cell depends on a cell system which includes the nucleoli and ribonucleic acid in the cytoplasm. Thus, since cellular growth consists largely in protein formation, growing cells are characterized by the presence of nucleoli and a cytoplasm giving the reactions for ribonucleic acid, as Thorell (1947*a*) demonstrated in red-cell formation by adult mammalian bone marrow. In this tissue he found that these criteria of growth disappeared from cells that contained only a trace of haemoglobin. Taking haemoglobin content as a measure of differentiation he concluded that growth and differentiation occurred in separate phases which overlapped only slightly.

In the circulating red blood cells of the chicken embryo investigations with methyl green-pyronin show that the pyronin staining nucleoli remain until the fourth day of incubation and until the cellular haemoglobin concentration reaches 9–11 %, which is more than one-third of concentration reached at hatching; pyronin staining of the cytoplasm persists longer still. On the assumption that pyronin staining indicates the presence of ribonucleic acid it is concluded that the capacity for growth is retained until the differentiation is more than one-third completed, so that in contrast with red-cell formation in the mammalian bone marrow, there is a considerable overlapping of an earlier phase of growth by a later phase of differentiation. It must be pointed out, however, that these results and conclusions apply only to the primitive red blood cells, which form more than 90 % of the cells circulating during the fourth day (Dawson, 1936). It was not possible to make similar observations on the definitive cells, because they do not predominate in the circulation until the characteristics of growth have disappeared. Therefore, it is not excluded that, in the definitive red cells, the relationship of cell growth to cell differentiation may differ from the relationship in the primitive cells and may resemble that demonstrated by Thorell (1947*a*) in the adult mammalian bone marrow.

Nevertheless, the considerable overlapping of the phases of growth and differentiation in the primitive cells may be of general significance, for it forms an exception to the generalization suggested by Thorell (1947*b*) that in tissue formation there is a considerable separation of phases of growth and differentiation. Many adult tissues possess, like the blood, systems for replacing cells lost in normal function or to form additional functioning tissue in response to appropriate stimuli. If in such systems a phase of growth is followed by a phase of differentiation the existence of a degree of overlap between the two phases might have important implications. If cells became 'fixed' at a stage of partial differentiation, while retaining the ability to grow, they would conform to many of the characteristics of malignant cells. It might be suggested that the extent of the overlap between the phases of growth and differentiation might affect the possibility of such cells arising.

Metabolism and red-cell development. There is evidence (O'Connor, 1951) that the oxygen consumed by the circulating red blood cells of the chicken embryo is used entirely in the catabolism of carbohydrate. Hence curve 5 in Fig. 1 indicates relative rates of carbohydrate catabolism as well as rates of oxygen consumption. Before the end of the seventh day this metabolic measurement undergoes considerable changes as do the measurements indicating embryological development, namely haemoglobin content, number of dividing cells and cell size. Interdependence of these processes of embryonic development and respiration (or carbohydrate metabolism) is suggested by the fact that all measurements, both developmental and metabolic, reach constant levels after the eighth day of incubation and very nearly at the same time (see Fig. 1).

Previously it has been suggested (see Brachet, 1947) that, in embryonic tissues, there is a fraction of catabolism specifically associated with the processes of development, and which can be distinguished from the 'maintenance' metabolism by which the cells are maintained in equilibrium with their environment. Such a distinction is suggested with regard to carbohydrate metabolism by the investigations of Needham & Lehmann (1937) and with regard to respiration by Moog (1944). If a special 'developmental' metabolism occurs in the circulating red blood cells of the chicken embryo it should be present on the fourth day of incubation, when development is proceeding rapidly, and absent after the ninth day, when development is complete or nearly so. Thus a comparison of the responses of cell respiration to metabolic inhibitors at these times might give information regarding the existence and the properties of a fraction of cellular catabolism specifically associated with the processes of embryonic development.

SUMMARY

1. The haemoglobin content has been measured in the circulating red cells of the chicken embryo from the end of the third day of incubation until hatching.

2. After increasing rapidly, the amount of haemoglobin per cell drops suddenly at the end of 7 days' incubation. This drop is ascribed to the replacement of primitive red cells by definitive.

3. The intracellular distribution of nucleic acid has been studied by staining with methyl green-pyronin. Changes in the distribution of ribonucleic acid indicated that growth ceased in cells in which the haemoglobin concentration was 9–11 %. From these findings it was concluded that, in the primitive red cells, there is a considerable overlapping of phases of growth and differentiation.

4. Developmental changes in the circulating red blood cells have been compared with changes in the rate of respiration. The significance of these changes has been discussed with regard to the possibility of a fraction of total catabolism being specifically associated with embryonic development.

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THE DEVELOPMENT OF THE HUMAN MANDIBULAR JOINT

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INTRODUCTION

The mandibular joint has long been of interest in that phylogenetically it represents a new joint replacing the older one between the articulare (malleus) and the quadrate (incus). In this connexion considerable controversy has arisen over the formation of the articular disc and its relation to the lateral pterygoid muscle. Furthermore, the mode of formation of the mandibular joint is somewhat different from that of most other synovial joints.

In the literature, so far as I have found, the only previous workers who studied the development of the human mandibular joint by means of a series of specimens are Kjellberg (1904), Vinogradoff (1910) and Mundaca (1948) The earliest stage described by any of these three workers is one of 35 mm. C.R. length.

A considerable period before there is any sign of a joint-cavity the chief elements of the mandibular joint have been mapped out. This is particularly true with regard to the condylar process of the mandible. Even as early as the 22 mm. stage in the human embryo there is some indication of its form.

MATERIAL AND METHODS

The materials used in this work were one embryo of 22 mm. C.R. length and a series of foetuses of 30, 34, 40, 48, 57 (two specimens), 60, 65, 70 (two specimens), 95, 150, 180 mm. (two specimens) C.R. length and one of full term. The material was fixed in formalin, dehydrated and cleared by routine methods and embedded in paraffin. One of the 57 mm. specimens, the 180 mm. and the full-term specimens, however, were treated by a double embedding method.

OBSERVATIONS

22 mm. stage

A considerable amount of bone-formation has taken place and formed a plate on the lateral side of Meckel's cartilage; this plate is in the substance of, and is surrounded by, that mesodermal condensation which outlines the mandible and precedes the formation of bone. The plate of bone is confined to the region of what approximately corresponds to the future body of the mandible, but the mesodermal condensation can be traced further backwards, always, of course, on the lateral side of Meckel's cartilage and the associated branches of the mandibular nerve. The condensation admittedly becomes gradually much less sharply defined but is still distinguishable from the surrounding tissue. Finally, some distance beyond the limit of bone-formation, the lateral pterygoid muscle can be seen running into and outlining the terminal part of the mesodermal condensation. This is the first indication of the condylar process in my series (Pl. 1, fig. 1). Meckel's cartilage with the inferior dental and lingual nerves on its upper surface lies medial to this rudimentary condylar area and inferior to the lateral pterygoid muscle.

30 *mm. stage*

The mesodermal condensation of the mandible is now everywhere clearly defined. In a section, the head of the condyle appears as a distinct oval-shaped mass to which the fibres of the lateral pterygoid are attached. Above and lateral to the condyle is an oval condensation, in the centre of which is a small area of bone-formation. This is the first indication of the temporal element of the articulation in my series. Already the outermost cells of the condylar condensation form a distinct layer and the temporal condensation shows the same feature but to a less marked extent. This is the first indication of the future layer of fibrous tissue which will cover the articular surfaces of the condyle and the temporal bone.

34 *mm. stage*

The tissue forming the condylar process has been invaded by an extension of the membrane bone from the ramus. A considerable proportion of the condylar neck and the anterior half of the head are occupied by this membrane bone, but there is only a slight indication of it in the posterior half of the head. In the early stages, a coronal section through the condyle head shows the zygomatic arch, since the condyle is well ventral to the temporal region compared with its definitive position (Pl. 1, fig. 2). There is a wide interval between the condylar and zygomatic condensations, which is filled with a loosely formed tissue apart from a dense strip lying close to the condyle. A few fibres of the lateral pterygoid muscle can be traced into the medial side of this strip of tissue. In the previous stage there is already an indication of this narrow condensation, also connected to the lateral pterygoid.

Traced farther back to the point where the condyle is decreasing in size, the zygomatic condensation changes its oval shape and bends medially as a horizontal shelf forming the anterior root of the zygoma and the roof of the future mandibular joint. Some bone had been formed in this condensation and it is continuous with the bone forming in the temporal region lateral to the middle ear. The distinction of the superficial layer of the condylar and zygomatic condensations noted at the previous stage has become more marked and the fibrillar element of this layer is now apparent.

40 *mm. stage*

Very few changes, except for a general increase in size, are to be seen compared with the preceding stage. There is a greater amount of membrane bone-formation in both the head of the condyle and the zygomatic condensation. There is the same arrangement of a wide area of loosely formed tissue and a narrow strip of denser tissue between the zygoma and the condyle, the future intra-articular region. On the medial side the connexion of this narrow strip with the lateral pterygoid is maintained (Pl. 1, fig. 3).

48 *mm. stage*

The secondary cartilage of the condyle is seen for the first time at this stage. It appears as a broad fringe of tissue at, and continuous with, the tip and lateral side of the core of membrane bone in the condyle, and is covered superficially by a thick fibrous layer, the deeper cells of which are continuously adding to the cartilage. At this stage the cartilage consists of large round cells which, in the one direction, can be traced in series with the cells of the fibrous-tissue covering which gives origin to them, and in the other direction are incorporated in the lacunae of the adjacent bone; that is, there is a continuous series from cells that are fibroblasts, through this cartilage-like tissue, to cells that are osteoblasts. The intercellular substance of the cartilage in the direction towards the fibrous-tissue covering it becomes less in amount and is continuous with its fibrillar network, while in the other direction it merges with the intercellular element of the bone.

57 mm. stage

The condylar cartilage is now in the form of a cone whose tapering end extends forwards into the ramus of the mandible. The innermost cells of the fibrous-tissue layer outlining the condyle have become more definitely flattened and spindle-shaped, and are arranged with their long axes parallel to the condyle surface. These cells, traced towards the centre of the condyle, gradually become rounded and merge into the area of cartilage (Pl. 1, fig. 4). Towards the anterior end of the condylar cartilage the cell-spaces have become larger, and the intercellular material appears as more sharply defined bars between the cells, as if it had been compressed.

At this stage there is the first sign of joint-cavity formation. This appears as a thinning-out of the loose tissue on either side of the strip of dense tissue above the condyle (Pl. 1, fig. 4). It is now obvious that this strip of tissue is the future articular disc; like the superficial layers of the condylar and zygomatic-temporal condensations, it is still quite cellular. A similar picture was found in another foetus of the same size. There is already a certain indication of a capsule as, from the lateral side of the zygomatic-temporal condensation, a narrow extension passes downwards to link up with the lateral margin of the future articular disc and reach towards the lateral side of the condyle.

60 mm. stage

Joint-cavity formation is somewhat advanced compared with the preceding stage. The strip of dense tissue above the head of the condyle is now readily identifiable with the articular disc as the lower joint cavity has appeared as a distinct cleft. This cleft-like lower joint-cavity is limited to the central area of the condyle. The formation of an upper joint-cavity does not appear to have progressed so far.

65 mm. stage

The condyle has greatly increased in size and is completely occupied by cartilage, apart from the area of proliferation of the cells of the fibrous-tissue covering. This area is now confined to the articular surface of the condyle, except for a noticeable extension into the lateral half of the condyle head. The larger size of the cartilage cells and the compressed appearance of the intercellular material, first seen at the 57 mm. stage, now affect the bulk of the cartilage, and, in the older more anterior part of the tissue, the cells show some degenerative changes, being shrunken and distorted.

Joint-cavity formation is considerably advanced, particularly in the lower joint-cavity. This is well formed over the whole of the upper surface of the condyle. The upper joint-cavity is confined to the posterior part of the articular region and does not extend medially and laterally to the same extent as the lower joint-cavity. Between the well-formed lower joint-cavity and the developing upper joint-cavity the articular disc is clearly defined, though in front of the area where formation of the upper joint-cavity has commenced it is still connected to the zygomatic region. With the marked increase in the size of the condyle, the temporal and condylar articular surfaces have been approximated, so that all sign of the wide area of loosely formed tissue which previously intervened between the superficial layer of the temporal condensation and the future articular disc has disappeared.

70 *mm. stage*

The same appearance in the mandibular element of the joint is to be seen at this stage, except for one important feature: this is the appearance of irruptions of vascular mesenchyme in the ramus part of the cartilage, which break down the tissue in their path (Pl. 1, fig. 5). The cartilage in this area has become calcified.

Both joint-cavities may be said to be completely formed though they are still crossed by a number of delicate strands of tissue connecting the articular disc to the respective articular surfaces. The bone in the temporal region is greatly increased in amount and has extended so as to form a roof for the joint—the glenoid fossa—wider than the articular surface of the condyle. Fibre-bundles are beginning to appear in the disc and the layers of tissue covering the temporal bone and the condyle. On the lateral side, indication of the capsule is confined to a linking up of the fibrous-tissue layers covering the temporal bone and the condyle with the substance of the articular disc. On the medial side this is obscured by the attachment of the lateral pterygoid to the condyle and the disc.

95 *mm. stage*

The breaking down of the condylar cartilage, first seen in the preceding stage, has become more extensive, the whole anterior two-thirds of the cartilage being invaded by more numerous and larger areas of vascular mesenchyme. In the temporal element of the joint, bone-formation has occurred to a marked degree at the posteromedial angle of this surface. The bone in this region, being of open texture and with such large cell-spaces, has almost the appearance of an area of secondary cartilage.

150 *mm. stage*

The process of breaking down of the condylar cartilage has continued so that, at this stage, the only part of the cartilage unaffected is that which occupies the upper half of the condylar head beneath the proliferating fibrous tissue; this tissue no longer extends into the lateral half of the condyle head, being confined to the area immediately below the articular surface. The vascular mesenchyme does not produce a total removal of the cartilage; instead, narrow trabeculae of the cartilage are carved out which are surrounded by wide marrow spaces. The more anterior of these trabeculae have become surrounded by new bone-formation, and the whole area is sharply outlined by the membrane bone of the ramus.

In the temporal region an area of secondary cartilage has appeared. It is in the same part of the joint surface as that which showed the extensive bone-formation in the preceding stage.

180 *mm. stage*

In the mandibular element of the joint a new feature has appeared. In the cartilage zone several wide channels are to be seen which pass through the cartilage from the covering layer of fibrous tissue and link up with the marrow spaces beneath the cartilage. These channels contain blood-vessels.

In the medial part of the temporal region of the joint secondary cartilage is still present. In a sagittal section from the medial part of the joint a distinct connexion between the articular disc and Meckel's cartilage can be seen, the disc being continuous with the tendon of the lateral pterygoid muscle (Pl. 1, fig. 6).

Full term

There is little difference between this stage and the preceding one except for an increase in size. In the condyle, however, the cartilage is reduced to an even narrower zone beneath the covering layer of fibrous tissue than at the 180 mm. stage. In the zone of cartilage the vascular channels, first seen at the 180 mm. stage, are still present. All trace of active growth of secondary cartilage has disappeared from the temporal region of the joint. There is no sign of the formation of an eminentia articularis, the bone forming the temporal surface of the joint being almost flat, except for a shallow concavity. The articular disc is composed of dense fibre-bundles. It is well vascularized peripherally but is avascular in its central part. At this stage it contains no cartilage cells, nor are there any such cells in the actual covering layers of the articular surfaces. Thus even the condyle may only be considered as being covered with fibro-cartilage if the secondary cartilage deep to the surface layer of fibrous tissue is considered.

DISCUSSION

The mandibular joint is primarily produced by the growth of the sclerogenous tissue of the developing mandible towards the corresponding tissue in the temporal region. This tissue first produces bone in both elements. These areas of bone, however, and the fibro-cellular condensations surrounding them, are always separated by a wide intra-articular fibrous-tissue filled interval. The final approximation of the mandible to the temporal surface in a dorsal and cranial direction is brought about by the development of the secondary cartilage in the condylar process. Thus the formation of the mandibular joint is considerably different from that of most other synovial joints in that the elements of the joint when first outlined in mesenchyme, and for some time after, are widely separated and are only finally approximated by the growth of a special tissue. This cartilage appears towards the 50 mm. stage and produces a marked increase in the size of the condyle between this time and the 65 mm. stage. By this rapid growth of the condyle, the previously wide intra-articular interval is largely obliterated. The only intervening tissue left is that strip of dense tissue immediately above the upper surface of the condyle which appears as early as the condylar condensation itself, and which is connected to the lateral pterygoid muscle from the beginning. This strip of tissue becomes the articular disc and would appear to be an extension of the tendon of the lateral pterygoid into the joint area. A similar conclusion was reached by Harpman & Woollard (1938), who decided that the medial portion of the disc, at any rate, arises from the tendon of the lateral pterygoid. Harpman & Woollard also demonstrated that at the 53 mm. C.R. length stage the tendon of the lateral pterygoid extended backwards to be attached to that part of Meckel's cartilage which becomes the malleus; this confirmed the similar observation of Kjellberg (1904) in a human foetus of 55 mm. Definite support for this has been obtained from my own series. In serial sagittal sections from the foetuses of 34 and 57 mm. C.R. length the tendon of the lateral pterygoid was traced beyond its attachment to the condyle of the mandible and reaching to Meckel's cartilage. This connexion was found also in the foetus of 180 mm. C.R. length (Pl. 1, fig. 6).

In the opinion of Vinogradoff (1910) the formation of the articular disc is intimately connected with the movement of the articular surfaces, especially of the condyle, towards one another, the tissue between the articular surfaces being compressed and so forming the disc. According to Vinogradoff it is only when the articular surfaces are closely approximated that the disc appears. However, it has been seen that the tissue of the future articular disc is quite distinct long before this approximation, thus ruling out the possibility of a direct formation of the disc by a compression of the intra-articular tissue. The formation of the joint-cavities on either side of this strip of tissue is associated, in time at least, with the great increase in the size of the condyle and its approach to the temporal element of the articulation.

I cannot support the observations of Mundaca (1948) that the elements of the mandibular joint have not appeared at 35 mm. and that they are only beginning to be outlined at the 46 mm. stage. In the 34 mm. specimen in my series, these elements are as well formed as Mundaca describes and figures them at the 46 mm. stage, and are assuming this appearance even at the 30 mm. stage.

It would appear that the lateral pterygoid is of importance in the definition not only of the articular disc but also of the condyle. At the 22 mm. stage the lateral pterygoid is running into and outlining the future area of the condylar process even though the mesodermal condensation for the mandible has not quite reached that level.

In the temporal element of the joint areas of secondary cartilage occur, thus paralleling the condylar element. These areas appear later, are much smaller in size than the condylar cartilage, and have disappeared before birth. Their appearance is localized and is apparently associated with a quite local and transient necessity for rapid growth.

SUMMARY

1. The development of the human mandibular joint has been studied in one embryo of 22 mm. C.R. length, and a series of fifteen foetuses from 30 mm. C.R. length to full term.

2. The elements of the human mandibular joint are fully sketched-out at

34 mm. C.R. length, having been indicated even earlier. The lateral pterygoid appears to assist in the outlining of the condylar element.

3. Joint-cavity formation has begun at 57 mm. C.R. length. Both joint-cavities are completely formed between 65 and 70 mm. C.R. length.

4. From its first appearance the tissue of the articular disc is connected to the lateral pterygoid muscle and seems to be an extension of the fibrous part of the muscle.

5. The lateral pterygoid muscle is connected through the medial part of the articular disc to the malleus. This connexion persists until at least 180 mm. C.R. length.

6. Areas of the temporal element of the joint pass through a secondary cartilage phase, but in contrast to the condylar cartilage, these areas are smaller in size, later in appearance, and have disappeared before birth.

I am indebted to Prof. J. D. Boyd for the loan of the 60 mm. C.R. specimen and to Dr J. H. Scott for the loan of one of the 180 mm. C.R. specimens.

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EXPLANATION OF PLATE

List of abbreviations

Cond.	Condyle of the mandible	*L.N.*	Lingual nerve
Sec.C.	Condylar secondary cartilage	*L.Pt.*	Lateral pterygoid muscle
F.C.A.	Future condylar area	*M.C.*	Meckel's cartilage
I.D.N.	Inferior dental nerve	*Zyg.*	Zygomatic arch

All the figures are of coronal sections through the head, except for fig. 6 which is a sagittal section.

Fig. 1. 22 mm. C.R. Showing the first indication of the area of the condyle of the mandible. Haematoxylin and eosin. ×90.

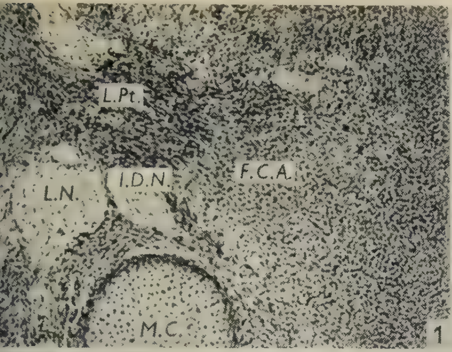
Fig. 2. 34 mm. C.R. Showing the main elements of the joint well mapped out. Note the connexion of the strip of tissue above the condyle of the mandible to the lateral pterygoid muscle. Mallory. ×55.

Fig. 3. 40 mm. C.R. Note the continued connexion of the strip of tissue above the condyle of the mandible to the lateral pterygoid muscle, and the wide interval between the condyle and the zygomatic arch. Mallory. ×55.

Fig. 4. 57 mm. C.R. Showing the first appearance of the joint cavities as a thinning-out of the tissue on either side of the strip of dense tissue above the condyle of the mandible (the future articular disc). The secondary cartilage which was first seen at the 48 mm. stage in the condyle is now of quite large size. Mallory. ×55.

Fig. 5. 70 mm. C.R. Showing the full extent of the carrot-shaped mass of the secondary cartilage of the mandibular condyle. The joint cavities are now well formed. Masson. ×20.

Fig. 6. 180 mm. C.R. Showing the connexion of the lateral pterygoid muscle through the articular disc to the malleus. Masson. ×10.



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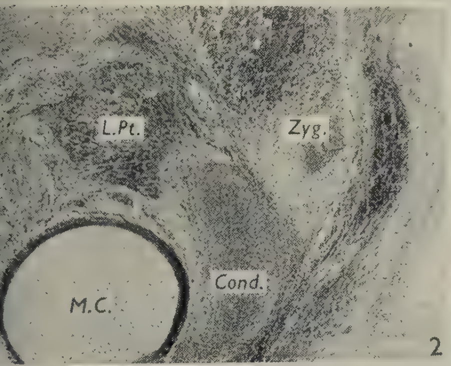

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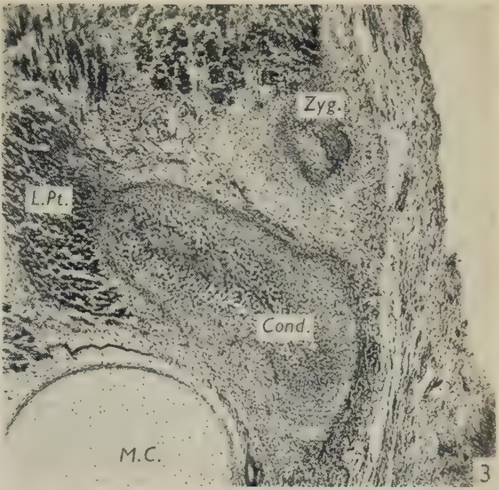

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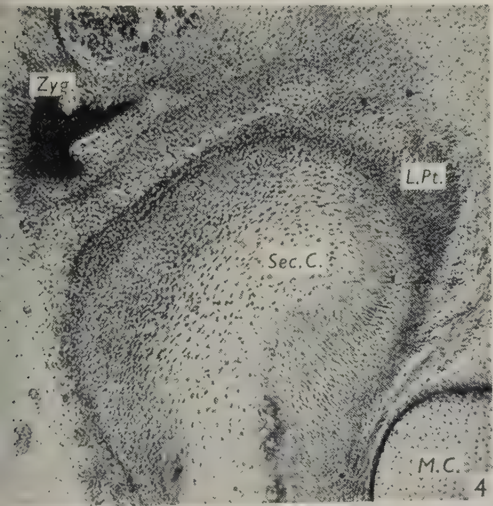

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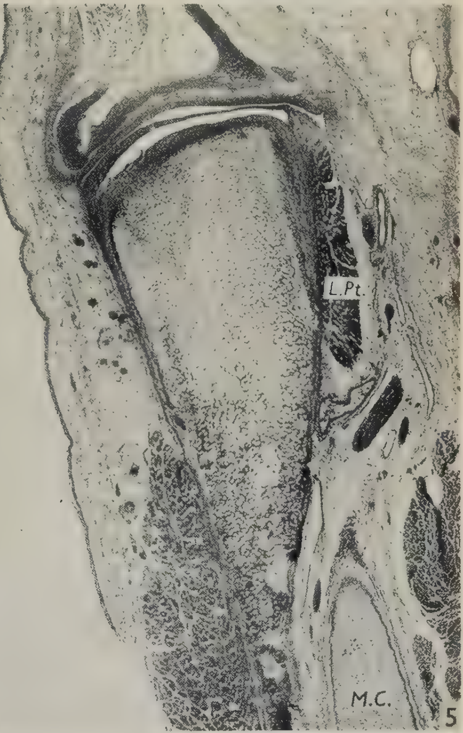

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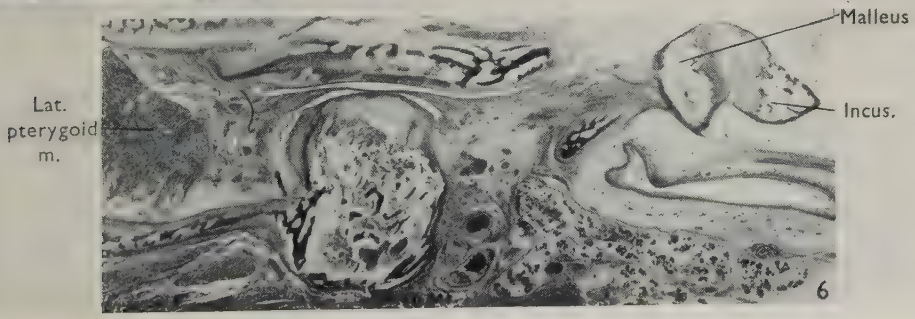


6

YMONS—The development of the human mandibular joint

REVIEWS

Topographische Ausdeutung der Bronchien im Röntgenbild mit Berücksichtigung der neuzeitlichen Nomenklatur. By CLAUS ESSER. (Pp. 152; illustrations; 12 × 8½ in.; DM. 33.) Stuttgart: Georg Thieme Verlag. 1951.

In this handsomely illustrated book, the author discusses at great length the anatomy of the bronchial tree as it appears to the radiologist. Each segment is considered separately and the previously expressed opinions concerning its site of origin, distribution and terminology are given. Unfortunately, the author does not sum up the evidence in favour of the nomenclature recently approved by the Thoracic Society after free discussion with many international representatives.

The book does not add appreciably to our knowledge concerning the correlation of the radiological appearances with the anatomy as obtained by bronchoscopy, casts and dissections. The quality of the bronchograms is very good and the work is obviously of importance to those interested in the finer details of the radiological anatomy of the bronchial tree.

G. SIMON

Der Einfluss des Nervensystems auf Bau und Tätigkeit der Geschlechtsorgane des Menschen. By H. STIEVE. (Pp. viii + 191; 93 illustrations; DM. 36.) Stuttgart: Georg Thieme Verlag. 1951.

In this volume Prof. Stieve summarizes and integrates his extensive and well-known investigations concerning the influence exercised by the nervous system on the human male and female reproductive systems. The facts recorded are based largely on the histological examination of testes and ovaries removed from individuals who had died after severe mental (as for example, in capitally convicted criminals) or physical stress. Such material obviously requires the most careful control and this, it would seem, has been attempted for the author writes of having 'viele Hunderttausende histologischer Schnitte durchmustert!' How heartfelt must be his wry reference to *magni formicae laboris*! Unfortunately, all of the illustrations are drawings and, although these are from the skilled hand of Neresheimer, the material deserved photomicrography. Indeed the absence of significant visual documentation detracts from the value of the work. Nevertheless, Prof. Stieve's recorded histological details represent an impressive contribution to our knowledge of the effects of psychic stress on the structure and activities of the ovaries and testes. Not all will be willing to follow him in his attempts at explanation of how the excitation of the central nervous system causes the alterations in the microscopic structure of the exocrine and endocrine components of the sex glands. In particular, the account of the nerve supply to the ovary will hardly receive universal acceptance for the nerve fibres in this organ are described as terminating in a *Grundplexus* which permeates its structure, including even the corpus luteum.

As is not surprising (for if the alleged phenomenon, in fact, occurs nervous influences on the ovary offer a possible explanation) Prof. Stieve has a long section on 'paracyclical ovulation'. This is the term he uses for ovulation in a given ovarian cycle over and above the normal mid-interval shedding of an egg. He produces clinical evidence in favour of such supernumerary ovulation, and if not convincing in itself, such evidence should be most helpful in preventing too rigid an adherence to fixed laws in a biological system.

It is a sad commentary on our time that in a book on human biology the author feels constrained to deny rumours about the sources of his research material. In his own words: 'Ich darf hier nochmals versichern, das ich niemals ein Konzentrationslager betreten und auch niemals die Leiche eines Menschen untersucht habe, der im Konzentrationslager verstorben war.' Or, again, but with a difference: 'alle unsere Nervenpräparate wurden durch Fliegerbomben zerstört.' Can such sentences, and their implications, be equalled in the recorded literature of biology? Prof. Stieve's book is more than a contribution to the study of the influences of the nervous system on the structure and activity of the human sex organs—it is a commentary, albeit an inadvertent one, on our times. J. D. BOYD

BOOK RECEIVED

Atlas of Human Anatomy. By F. FROHSE, M. BRÖDEL and L. SCHLOSSBERG. Explanatory text by J. F. Williams, new edition. (Pp. 88; 16s.) London: George Allen & Unwin Ltd. 1952.

THE ATTACHMENTS AND FUNCTION OF THE ABDUCTOR POLLICIS BREVIS

By J. R. NAPIER

Department of Anatomy, St Thomas's Hospital School of Medicine, London

It has become apparent, through the clinical investigation of the long-term results of peripheral nerve injuries, that minor disturbances of function at the metacarpo-phalangeal joint of the thumb have a disproportionately grave effect on the function of the hand as a whole (Bowden, 1951), and in particular on fine grasping movements between the thumb and the digits.

The extent and importance of the movements normally occurring at this joint do not appear to be generally recognized, in spite of the comprehensive accounts given by Duchenne (1856) based on electrophysiological experiments, and more recently by Bunnell (1938). Both these authors describe the movements of abduction, rotation and flexion that occur at this joint. Duchenne (1856) states that the muscle responsible for these movements is the abductor pollicis brevis and he cites the observations of Sabatier (1764) and Soemmerring (1796) in support of this view. Both these authors describe the insertion of the muscle into the dorsal expansion as well as into the base of the proximal phalanx. In more recent times this double insertion has received scant regard, the portion passing to the dorsal expansion being dismissed as a slip. A preoccupation with the action of the muscle on the carpo-metacarpal joint has tended to eclipse its primary action on the metacarpo-phalangeal joint. In his Croonian Lectures, Beevor (1904) describes the action of the abductor pollicis brevis on the metacarpal but not on the proximal phalanx.

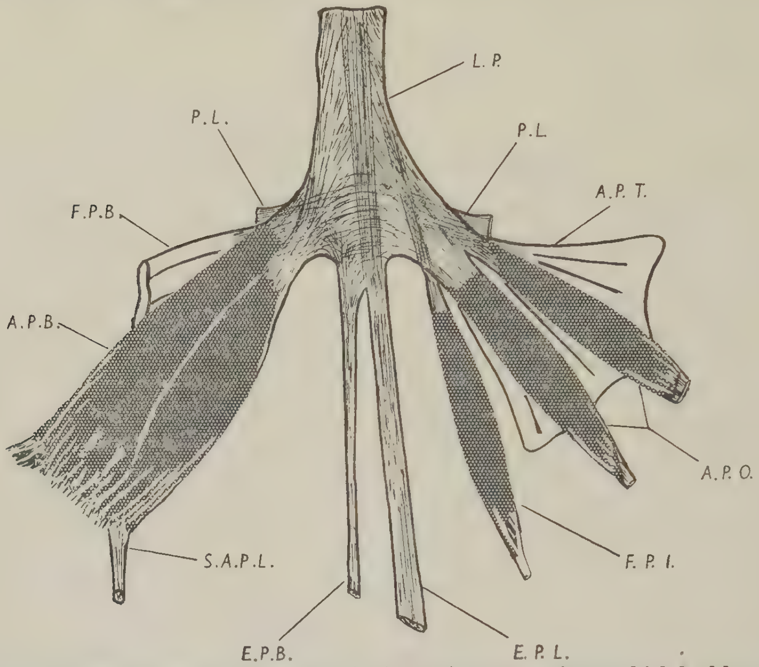
The present investigation was undertaken to amplify the observations of Duchenne (1856) and to correlate the anatomical findings with the clinically observed disturbance of function resultant upon the loss or impairment of metacarpo-phalangeal joint movement.

The following account is based on the dissection of the muscle in forty-two subjects including seven post-mortem specimens, thirty-four formalin-fixed cadavers and one amputation specimen. The clinical material consisted of sixty consecutive cases of median nerve injury.

OBSERVATIONS

The form of the muscle

The abductor pollicis brevis, which has a fusiform outline, is usually incompletely divided into two parts; the plane of separation passes obliquely so that the larger medial part slightly overlaps the smaller lateral part (Text-fig. 1). Some variation occurs in the relative proportion of the two bellies which are loosely connected by a small amount of areolar tissue and may easily be separated as far proximally as their origin. The bipartite nature of the muscle is reflected in the independent formation of the proximal parts of the common tendon, that of the lateral head appearing on the superficial surface of the muscle while the tendon of the medial head lies on its deep surface. In extremely muscular subjects the typical appearance may be obscured, but where the muscle is poorly developed, or in cases of extreme wasting following peripheral nerve injury, the essential tendinous arrangement of the two portions is clearly shown.



Text-fig. 1. The dorsal expansion of the thumb, drawn from a specimen which had been dissected away from the underlying bone and spread out flat. The muscles shaded-in are inserted wholly or partly into the expansion. *A.P.B.* abductor pollicis brevis; *A.P.O.* oblique head of adductor pollicis; *A.P.T.* transverse head of adductor pollicis; *E.P.B.* tendon of extensor pollicis brevis; *E.P.L.* tendon of extensor pollicis longus; *F.P.B.* flexor pollicis brevis. *F.P.I.* 1st palmar interosseous. *L.P.* longitudinal prolongation of lateral side. *P.L.* palmar metacarpal ligament; *S.A.P.L.* slip from abductor pollicis longus.

Attachments of the muscle

The origin of the muscle from the flexor retinaculum, the tubercle of the scaphoid and the crest of the trapezium is well known. An additional slip of origin arising from the tendon of the abductor pollicis longus and running into the lateral belly of the muscle is an almost constant finding, and was present in sixteen out of seventeen specimens in which this particular feature was noted. Frequent small slips of origin from palmaris longus and an occasional muscular slip from the skin over the thenar eminence were also noticed.

The insertion is by a flat tendon partly into bone and partly into the side of the extensor pollicis longus tendon, forming the major part of the dorsal expansion on the lateral side. The expansion consists of tendinous fibres some passing horizontally towards the dorsum of the digit, others running distally to form a longitudinal prolongation which fuses with long extensor tendon close to its insertion (Text-fig. 1). This longitudinal prolongation was well developed in only four dissections, in the remainder it was either absent or poorly defined and closely adherent to the shaft of the proximal phalanx. The horizontal portion, in addition to receiving fibres from abductor pollicis brevis, also receives reinforcing fibres from the flexor pollicis brevis and the palmar ligament of the metacarpo-phalangeal joint. Many of these fibres cross the tendon of extensor pollicis longus, superficially, to become continuous with the horizontal fibres of the expansion on the medial side, forming an aponeurotic sheath for the long extensor (Text-fig. 1). The form of the dorsal expansion of the thumb is altogether more simple than its homologue on the other digits.

The *medial* belly of the abductor pollicis brevis is inserted by the tendon on the lateral side of the base of the proximal phalanx. A few superficially placed fibres continue into the expansion, passing deep to those of the lateral belly. When the longitudinal prolongation is well marked some fibres from the medial belly may reinforce its anterior free edge. The fibres of the *lateral* belly have no bony insertion but fan out from the tendon to gain attachment to the extensor pollicis longus along a variable extent of its length, the inner fibres constituting the longitudinal element of the expansion and the remainder forming the horizontal portion; some of the latter curve sharply backwards and become continuous with the arching fibres of insertion of extensor pollicis brevis to form the sharply defined, sickle-shaped, posterior free border of the expansion.

The horizontal portion is reinforced on its deep surface by a few of the superficial fibres of insertion of flexor pollicis brevis, which lies somewhat medially and on a deeper plane than abductor pollicis brevis. A sesamoid bone on the lateral side of the metacarpo-phalangeal joint is an almost constant finding and is related to the capsule on the deep surface of the flexor (Joseph, 1951*a*).

Action of abductor pollicis brevis

The relation of the abductor pollicis brevis to the long axis of the metacarpal clearly indicates that it is mechanically capable of producing, first, abduction of the proximal phalanx by virtue of its insertion on the radial aspect of the bone and, secondly, medial rotation about a vertical axis through its effective attachment to the dorsum of the proximal phalanx through the expansion. This double action is reflected in the bipartite form of the muscle. Flexor pollicis brevis, being attached near the anterior aspect of the proximal phalanx and, therefore, slightly in front of the vertical axis of rotation, is in a position to flex the metacarpo-phalangeal joint and to produce some rotatory movement by virtue of the few fibres, already discussed, that reinforce the horizontal part of the dorsal expansion.

Experiments were carried out in the seven post-mortem subjects to demonstrate the action of the abductor pollicis brevis. With the first metacarpal fixed in abduction, traction in the line of the muscle fibres of the *lateral* belly produces both abduction and medial rotation of the proximal phalanx. Traction of the *medial* belly results principally in abduction, but in addition some very slight rotatory movement usually occurs. These observations have been confirmed by electrical stimulation on living subjects using an induced current from a Palmer coil.

These anatomical observations give support to the clinical inferences regarding the action of the muscle. In sixty consecutive cases of median nerve injury the commonest cause of loss of function, as judged by the ability to maintain intimate pulp-to-pulp apposition of the thumb and index finger, was due to a loss of the movements of abduction and medial rotation at the metacarpo-phalangeal joint. This defect was present in thirty-four instances, in all of which the abductor pollicis brevis was either weak or paralysed and the flexor pollicis brevis, the only other muscle attached to the lateral side of the base of the proximal phalanx, was clinically normal. These observations indicate that it is the abductor pollicis brevis which is normally responsible for these movements. The slight rotatory action of flexor pollicis brevis observed experimentally has not been demonstrated clinically.

Muscle attachments on the medial side of the proximal phalanx

The antagonists of the abductor pollicis brevis are the muscles inserted on the medial side of the proximal phalanx and into the dorsal expansion, namely, the adductor pollicis and the first palmar interosseous. The manner of insertion of these two muscles has been examined in fourteen dissecting room subjects, and since the findings differ from the usual description, they are recorded in some detail.

The whole of the transverse head and part of the oblique head of adductor pollicis is inserted into a tubercle on the medial side of the base of the proximal phalanx. At its insertion it is related to a sesamoid bone (Joseph, 1951*a*). The remainder of the oblique head is very variable. One or more slips arising from the lateral and antero-lateral aspects of the second metacarpal, and forming, therefore, the deepest portion of the adductor muscle, are inserted directly into the dorsal expansion without reaching bone (Text-fig. 1). One slip not infrequently joins the tendon of the first palmar interosseous to form the longitudinal prolongation of the dorsal expansion.

The 1st palmar interosseous, a muscle previously described by British anatomists as the deep head of the flexor pollicis brevis, varies considerably in size and importance, although its complete absence was noted in one case only. It arises from the anterior aspect of the first metacarpal near its base under cover of the 1st dorsal interosseous, and is inserted by a slender tendon into the medial side of the dorsal expansion. A direct bony insertion was not observed in any of the dissections. This observation is in accord with the mode of insertion of the rest of the palmar series which, according to Salsbury (1937), are inserted exclusively into dorsal expansion of the digits. The tendon of the first palmar interosseous, which passes deep to the horizontal portion of the fanning out from the oblique head, constitutes either a part or the whole of the anterior free border of the longitudinal prolongation.

DISCUSSION

The anatomical and clinical findings on which this paper is based are substantially in agreement with those of Duchenne (1856). Such disagreement as exists concerns relatively minor points. Duchenne credits the flexor pollicis brevis with a greater share in the formation of the dorsal expansion and its longitudinal prolongation than is usually the case. The rotatory function of the short flexor suggested by Duchenne is not apparent clinically nor is there sufficient anatomical evidence to make this movement even a theoretical probability. In pursuance of a functional homology between the extensor mechanisms of the thumb and the digits, Duchenne suggests that the attachments of both the abductor and flexor brevis to the longitudinal prolongation of the dorsal expansion provide a means by which the terminal joint is extended during opposition of the thumb. It is true that the action of extensor pollicis longus on the terminal joint may be simulated under certain conditions by the abductor pollicis brevis. This so-called trick movement is commonly seen in the presence of a complete radial nerve palsy (Jones, 1919) and may lead to the erroneous diagnosis of an incomplete lesion. The meagreness of the longitudinal prolongation on the lateral side, however, and its frequent adherence to the phalanx precludes the possibility, in most cases, that the extension of the terminal joint is due to an active pull through the dorsal expansion. Full extension can be produced, however, if the actions of the abductor and flexor pollicis brevis are imitated by forcibly flexing and abducting the proximal phalanx in a cadaver. As a result of flexion, which produces a greater prominence of the knuckle, and abduction, which further lengthens the course of the tendon, the tension in the extensor pollicis longus is increased and the terminal phalanx is pulled into extension. The flexor pollicis longus, being relaxed in flexion of the metacarpo-phalangeal joint, does not oppose the motion. Such a mechanism can play little part in extension of the terminal phalanx in the normal living limb, for adjustments in the tone of the extensor muscle belly fully compensate for any increase in the tension in the tendon. When the muscle is paralysed, however, the tendon acts, in effect, as a non-extensible ligament passing over the metacarpo-phalangeal joint.

Opposition of the thumb is essential to the performance of all grasping or pinching movements, which require the reciprocal pressure of two or more opposed digits. The value of this movement is twofold. First, it ensures that the whole of the available pulp surface of the digits is utilized, thus supplying the necessary friction and allowing the maximum sensory awareness of the object held. Secondly, it permits the clamp-like action of the opposing digits to be employed to the best advantage for purposes of stabilization. There is essentially only one position of the thumb that satisfies these requirements, namely, when it is fully abducted in a plane at right angles to the palm and fully rotated so that the palmar surface makes an angle of approximately 45° to the plane of the palm. Each digit can be brought into intimate pulp-to-pulp contact with the thumb held in this position. The abduction and rotation movement of the thumb takes place at both carpo-metacarpal and metacarpo-phalangeal joints (Pl. 1, fig. 1). As Haines (1944) has shown, the thumb is fully opposed when the carpo-metacarpal joint is fully flexed. The degree of this flexion, however, varies from one individual to another as does also the degree of flexion at the metacarpo-phalangeal joint (Parsons, 1895; Joseph, 1951 *b*). A reciprocal relationship probably exists between the flexion ranges at these two joints, for despite wide individual variations, the tip of the fully opposed thumb generally lies in approximately the same antero-posterior plane as the middle finger. When performing grasping actions the thumb maintains a constant position of abduction and rotation relative to the palm. Except for minor differences in the degree of flexion and extension at the three thumb joints, the fundamental position of the thumb is unchanged whatever the size or shape of the object grasped (Pl. 1, figs. 2–4). This is in fact the position of function of the thumb in grasping, and constitutes true opposition which must be regarded as a compound movement taking place at the carpo-metacarpal, metacarpo-phalangeal and interphalangeal joints.

True opposition, and thus the grasping mechanism, is profoundly disturbed by a paralysis of the abductor pollicis brevis, for, although abduction and rotation of the metacarpal are still possible, abduction and rotation of the proximal phalanx are abolished (Pl. 1, fig. 5). Without the latter movements intimate pulp-to-pulp apposition of the digits cannot be achieved, and hence all grasping actions of the hand are severely impaired.

SUMMARY

The abductor pollicis brevis was studied by dissection in forty-two subjects. Clinical evidence was drawn from sixty consecutive cases of median nerve palsy. The following conclusions were reached:

1. Abductor pollicis brevis, which is bipartite in form, is inserted partly into bone at the base of the proximal phalanx, and partly into the dorsal expansion. Its function is to bring about abduction and medial rotation at the metacarpo-phalangeal joint during the compound movement of opposition.
2. The antagonists to the abductor pollicis brevis are the muscles attached to the medial side of the proximal phalanx and to the dorsal expansion, namely, the adductor pollicis and the 1st palmar interosseous.
3. Paralysis of abductor pollicis brevis results in the loss of the abduction and rotation movements at the metacarpo-phalangeal joint, although movements at the carpo-metacarpal joint are undisturbed. In these circumstances opposition is incomplete, true pulp-to-pulp contact of the digits cannot be achieved, and consequently the grasp is severely impaired.

I wish to thank Mr Charles Gray, F.R.C.S., for the facilities accorded me at the Peripheral Nerve Injury Unit, Royal Free Hospital, and Prof. R. E. M. Bowden and Prof. D. V. Davies for the interest they have shown and the help they have given me in this investigation.

The photographs were taken and prepared by Mr A. L. Wooding of the Photographic Department, St Thomas's Hospital.

Fig. 5 of the Plate is reproduced with the kind permission of the editor of the *British Journal of Physical Medicine*.

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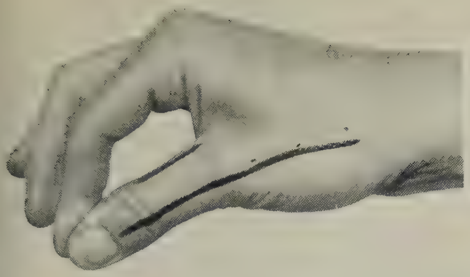

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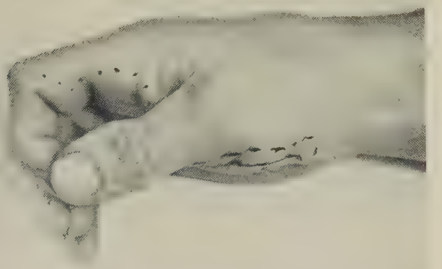

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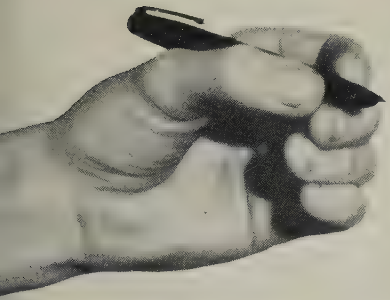

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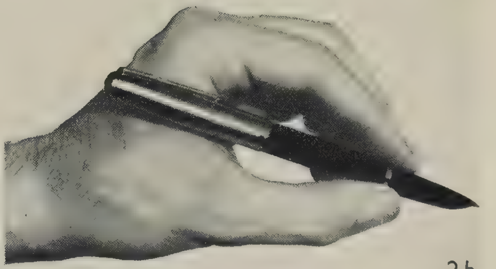

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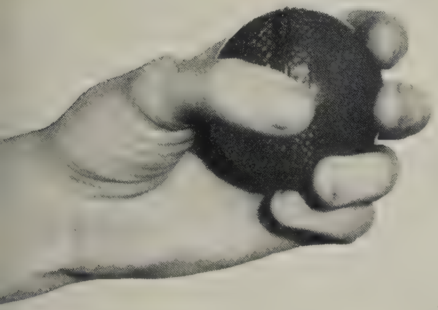

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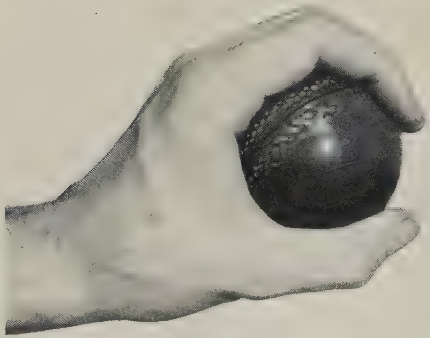

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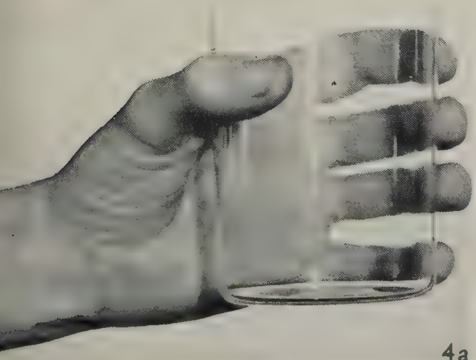

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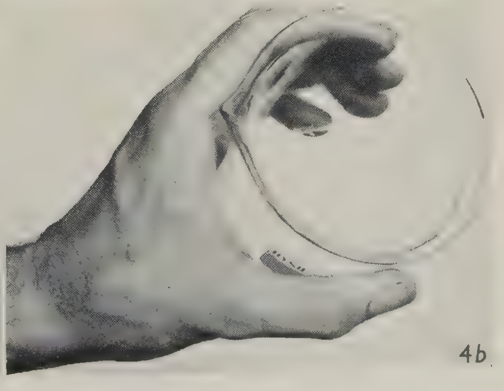

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APIER—ATTACHMENTS AND FUNCTION OF THE ABDUCTOR POLLICIS BREVIS

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EXPLANATION OF PLATE

Fig. 1. Normal opposition between thumb and index finger. Abduction and medial rotation are apparent at metacarpo-phalangeal joint of the thumb (compare Pl. 1, fig. 5).

Figs. 2–4. Anterior and lateral views of a normal hand showing opposition of the thumb during the performance of three types of grasping movement. Apart from slight adjustments of flexion and extension at the three joints, the thumb maintains a constant posture irrespective of size or contour of the object grasped.

Fig. 5. Attempted opposition between thumb and index finger in a patient with paralysis of abductor pollicis brevis. Note absence of abduction and medial rotation at metacarpo-phalangeal joint of thumb.

THE DEVELOPMENT OF MOUSE VIBRISSAE *IN VIVO* AND *IN VITRO*

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INTRODUCTION

For the analysis of genetic and environmental effects on hair growth, the behaviour of different types of hairs or follicles in any one individual or species is of particular importance. In this study, therefore, our attention was turned from the body hairs of mice to the specialized vibrissae on the face, in order that the two types might be compared *in situ* and in explants of skin cultivated in a nutrient medium.

Vibrissae, also known as whiskers, feelers or tactile, sensory or sinus hairs, are defined by Danforth (1925*a*) as 'large stiff hairs that are pre-eminently sensory'; they are distinguished by him from all other types of hair by the presence of erectile tissue in their follicles. The early histologists usually studied the innervation of vibrissal follicles without describing the other features in detail. Botezat (1897) pointed out the main differences between pelage and vibrissal hair follicles, and reviewed earlier literature. Vincent (1913) described the tactile hairs of the white rat, and Szymonowicz (1936) distinguished between the vibrissal follicles of various species within each mammalian order by the arrangement of blood sinuses.

Most studies of follicle development are restricted to the pelage. Maurer (1892) described two early stages of the development of vibrissae in the mole, and Zietzschmann (1920) defined the early stages in the pig. Grüneberg (1943*a*, *b*) noted that in the mouse embryo vibrissal follicles appeared earlier and developed faster than pelage hair follicles, and Falconer, Fraser & King (1951) described the relation of derma to epidermis in the first two stages of vibrissal follicle development in this species. To our knowledge, no account has been given of all the stages of the development of vibrissae in any mammal or of their relation to age.

In brief notes, Strangeways (1931) and Murray (1933) reported some development of vibrissal follicles in tissue cultures of guinea-pig and rat skin respectively. Hardy (1951) observed the complete differentiation of vibrissal follicles in living, unstained explants of upper lip skin from embryonic mice, but made no detailed study of the rate of development.

In this paper an account is given of the structure of vibrissal follicles in the adult mouse and of their development in the skin of the embryo. This is followed by a description, based on both living explants and serial sections, of the development of vibrissal follicles in tissue culture. The latter extends and amplifies the observations of Hardy (1951), and enables a detailed comparison with the development *in vitro* of pelage hair follicles (Hardy, 1949).

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* Guest worker in the Tissue Culture Laboratory, University of Texas Medical Branch, Galveston, at the commencement of this study.

MATERIAL AND METHODS

At Galveston the mice were supplied by Prof. R. F. Blount from his inbred strain of albinos at the University of Texas Medical Branch. Subsequently, in Sydney, another inbred albino strain was made available at the McMaster Laboratory. The age of embryos was determined as accurately as possible, that is, to the nearest day, by the recording of vaginal plugs and by comparison with Grüneberg's (1943*a*) description of external features. The ages of other mice are indicated in days after birth, preceded by a plus (+) sign.

The normal development of vibrissal follicles was studied in embryos at successive days of gestation from the tenth day until birth, and in a few young and adult mice. The main series of specimens was obtained at the McMaster Laboratory. Pieces of tissue including the upper lip were fixed in Zenker's fluid with 3 or 5% acetic acid, sectioned serially at 8 μ and stained with Mayer's haemalum, picric acid and eosin. The vibrissae with their follicles were dissected from the fresh skin of the upper lip of newborn, 3-day-old and adult mice, and whole mounts were prepared by fixation in 10% formalin and staining with carmalum or Delafield's haematoxylin.

In the preliminary experiments, numbered 1 to 3, which were performed at the University of Texas Medical Branch, and will be referred to as the Galveston series, the skin from the upper lip of 11-, 14- and 15-day embryos was cultivated in hanging drops by a method which has already been described (Hardy, 1949, 1951). Each explant included the locus of part or the whole of a row of vibrissal follicles, or of several rows. The medium consisted of equal parts of fowl plasma and an extract of a 7- to 13-day chicken embryo, and was renewed every 3 or 4 days. These explants were used primarily for a photographic study of the development of living follicles reported elsewhere (Hardy, 1951). For the present study daily records were made of living follicles, and after 5–14 days of cultivation the explants were fixed in Zenker's fluid with 3% acetic acid, sectioned serially and stained, as for embryo skin.

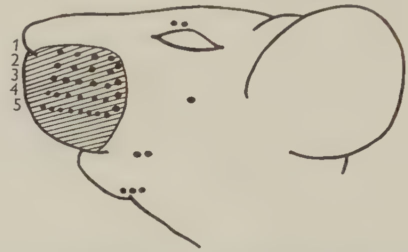
Exps. 4–7, to be known as the Sydney series, were carried out at the McMaster Laboratory. Each explant from a 12- or 13-day embryo contained three or four follicle rudiments from one row. It was placed, derma downwards, on the surface of a clot made from fowl plasma and chicken-embryo extract. Except for a 15-day embryo used in Exp. 6, all the chicken embryos were 10–13 days old. The medium was renewed every 3 or 4 days. Each day the explants were sketched, but not photographed. Cultivation was continued for periods up to 10 days, and all the living explants were fixed, sectioned and stained at suitable intervals.

For each series of cultures, litter-mate embryos were sectioned to determine the initial stage of development. The Sydney series of experiments provided suitable material for the analysis of rates of development *in vitro*, but the data from the Galveston series were omitted from this analysis because of the rather slow rates, probably due to the extra handling required for photography.

STRUCTURE AND DEVELOPMENT OF VIBRISSAL FOLLICLES *IN VIVO*

(1) *Position and arrangement of follicles*

The position of the principal facial vibrissae of the adult mouse is shown in Text-fig. 1. On the upper lip a vertical row of four large follicles alternates with five horizontal rows, in which the follicles vary in number. In rows 1 and 2 there are always four large follicles, and five in row 3. There are always at least six and eight follicles, graded in size, in rows 4 and 5 respectively. A variable number of vibrissal follicles is interspersed among those of the pelage at the anterior end of rows 4 and 5. The position and arrangement of the other vibrissal follicles of the face agrees with the descriptions by Danforth (1925*b*) and Grüneberg (1943*b*).



Text-fig. 1. Diagram of head of adult mouse, in which the position of each principal vibrissal follicle is indicated by a dot. Smaller vibrissal follicles are situated within the shaded area.

(2) *Structure of follicles in adult*

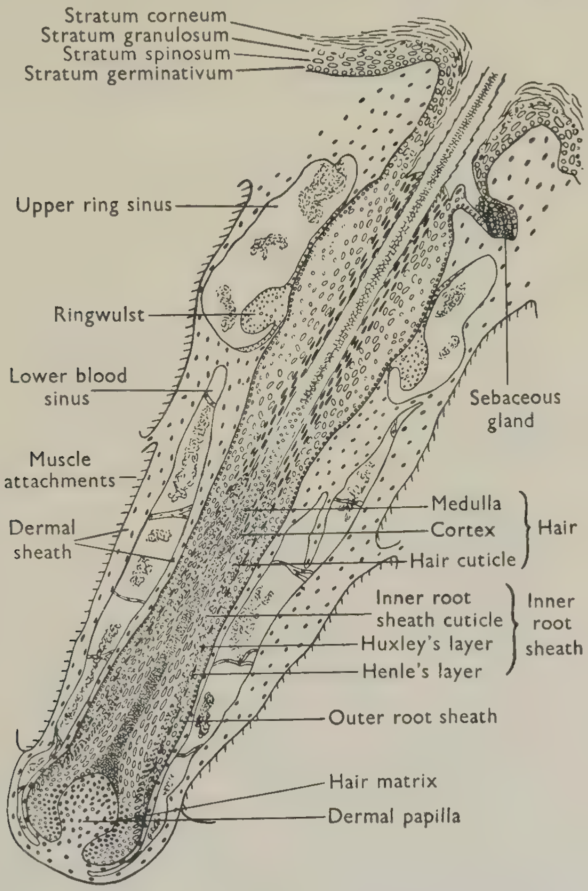
All hair follicles have the same basic structures, namely the epidermal hair matrix, outer root sheath, inner root sheath and hair, and a dermal papilla. These structures have been described in detail for man (Maximow & Bloom, 1948). Certain structures such as sebaceous glands, sweat glands, muscle attachments and a dermal sheath may also be present. All vibrissal follicles possess blood sinuses and abundant nerve endings which distinguish them from pelage hair follicles. The differences between these two follicle types in the mouse may be seen by comparing Text-fig. 2 with a diagram of an active pelage hair follicle (e.g. Hardy, 1949, text-fig. 1).

The follicles differ in size and shape. Pelage hair follicles are long and thin, and of rather uniform diameter. The vibrissal follicles which produce longer and thicker hairs, are themselves larger and stouter, and, due to two thickenings of the outer root sheath, have an hour-glass shape. The outer root sheath consists of a single outer layer of basal cells, together with three or four more layers of epidermal cells (Pl. 1, fig. 1). As in other rodents (Szymonowicz, 1936) this sheath is enlarged at a superior and an inferior swelling, in which the cells have increased in size.

At the level of the sebaceous gland, the vibrissa is anchored by the most distal cells of the inner root sheath, which forms a tight-fitting collar with a serrated inner margin (Pl. 2. fig. 11).

In adult mice, both vibrissae and pelage hairs are medullated. In the pelage the medulla cells of the follicle, and the resulting air spaces in the hair, are arranged in regular rows. This is the discontinuous type of medulla according to the classification of Hausman (1924). On the contrary, the medulla of vibrissae (Text-fig. 2) has cells and air spaces resembling a honeycomb, and is probably of the intermediate type (Hausman, 1924).

The dermal sheath of connective tissue surrounding the pelage hair follicle is insignificant, but a very thick sheath surrounds the vibrissal follicle. Within this sheath lie the blood sinuses, which are characteristic of all tactile hair follicles, but are absent from pelage hair follicles. The lower or venous blood sinus surrounds the deeper portion of the follicle, and its cavity is crossed by connective tissue bands.



Text-fig. 2. Diagram of active vibrissal follicle in adult mouse.

The cavity of the more superficial upper or ring sinus has no trabeculae; it contains the Ringwulst, also known as the pulvinus or annulus, which is attached to the dermal sheath adjacent to the follicle wall, and forms a collar surrounding the follicle. The Ringwulst apparently consists of connective tissue, similar to that of the dermal sheath, and is penetrated by nerve branches and blood capillaries.

In the mouse, the dermal papillae of the pelage hair follicles apparently lack a blood supply, but the papillae of vibrissal follicles contain blood capillaries. Vibrissal follicles are more richly innervated than those of the pelage, but the details were not studied. The vibrissal follicle has no smooth muscle corresponding to the arrector pili muscle of the pelage hair follicle, but it can be moved by a group of larger striated muscle fibres which are attached to the dermal sheath on one or other side of the follicle (Text-fig. 2). In the mouse, each vibrissal and pelage hair follicle has one small sebaceous gland, but no sweat gland.

(3) *Development of follicles*

The time-relations in histogenesis of the vibrissal follicles of the mouse have not been examined previously. Table 1 indicates that development is more rapid than in pelage follicles. The epidermal plugs of the first vibrissal follicles appear in the 12-day embryo, and hairs emerge 5–6 days later. Pelage hair follicles begin to form in the 14-day embryo, and require 9–10 days for the hairs to emerge.

Table 1. *Summary of stages of development of vibrissal and pelage hair follicles in mouse*

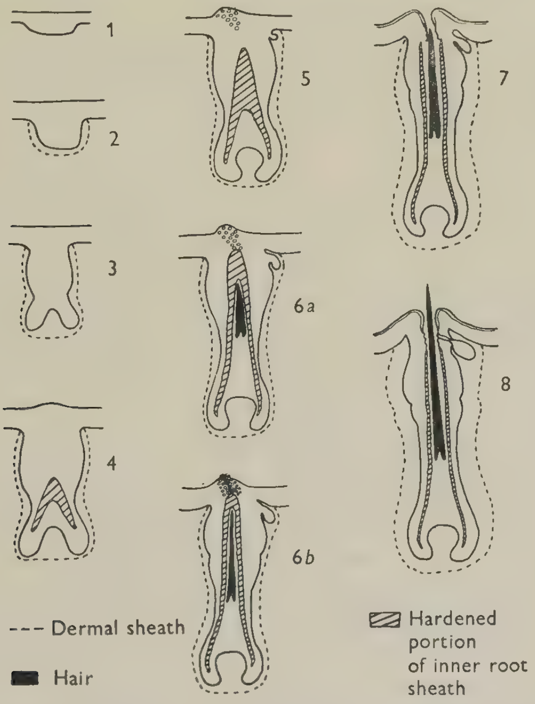
Stage no.	Degree of differentiation	Age (days)	
		Vibrissae	Pelage
0	No follicles	10–11	12–13
1	Follicle plugs	12	14
2	Pre-papillae	13	15
3	Papillae	14	16
4	Hair cones	14–15	19
5	Hair canals	15	20
6*a*	Hair formation	15–16	21
6*b*	Opening of hair canals	16–17	
7	Hairs in hair canals	17	+1
8	Hairs emerged	17–18	+2 to +3
9	'Trio' follicle groups	—	+4
10	Development of secondary follicles	—	+5 to +8
11	End of follicle length growth	—	+14
12	First resting phase	—	+18
13	Beginning of second growth cycle	—	+28

The differentiation of vibrissal follicles (Text-fig. 3; Pl. 1, figs. 2–5; Pl. 2, figs. 6, 7) is basically the same as that of pelage hair follicles (Hardy, 1951, text-fig. 2), which has already been described (Hardy, 1949). An epidermal downgrowth (stage 1), and a dermal papilla (stage 3) are formed. A hollow cone (stage 4), which gives rise to the inner root sheath, develops by the hardening of certain cells from the hair matrix. As this cone extends upwards, a hair canal (stage 5) is formed in the epidermis above the follicle. A keratinized hair (stage 6) appears inside the cone, pierces the cone (stage 7), and emerges from the skin (stage 8) through the open hair canal.

From the earliest stages the vibrissal follicles are larger and stouter than those of the pelage. The epidermal plug is surrounded by a dermal sheath even at stage 1 (Pl. 1, fig. 2). The follicle assumes the characteristic hour-glass shape as it elongates and as the dermal papilla is formed (Pl. 1, fig. 3). The thickening of the outer root sheath is evident at this stage, and by stage 6 the superior and inferior swellings have differentiated (Pl. 2, fig. 6).

In the vibrissal follicle the swelling which marks the position of the sebaceous gland is first seen at stage 4 at 14–15 days (Pl. 1, fig. 4), and the gland cells are differentiated at stage 6*a* at 15–16 days. The corresponding swelling in the pelage hair follicle is formed at stage 5 (20 days), and the sebaceous cells appear at stage 7 (+1 day).

In the development of a vibrissa, the first indication of a hair canal at stage 5 (Pl. 1, fig. 5; Pl. 2, fig. 8) is the condensation of cells, with strongly basophilic nuclei, on an elevation or cap of epidermis above the follicle. At stage 6*a* this condensation extends downwards to the tip of the hair cone (Pl. 2, figs. 6, 9). At stage 6*b* a canal has formed within this area by the keratinization of cells in the centre (Pl. 2, fig. 10). The canal extends from the surface of the epidermis to the tip of the inner root sheath, and is widest at its outer end, where keratinization probably began. At stage 7 the hair has pierced the inner root sheath to reach the open hair canal (Pl. 2, fig. 11), and at stage 8 it has emerged above the skin (Pl. 2, fig. 7). The development of hair canals in pelage hair follicles differs from that in vibrissae, and begins with keratinization within the epidermis at the level of the stratum spinosum (Hardy, 1949). In pelage hair follicles there is no cap of epidermal cells at stage 5, and no condensation of basophilic nuclei at the site of canal formation.



Text-fig. 3. Diagram of stages 1–8 in development of vibrissal follicles in mouse.

The thick dermal sheath, first seen at stage 1 (Pl. 1, fig. 2), surrounds the developing vibrissal follicle, and at stage 8 in 17- and 18-day embryos, a few isolated blood cells are visible within it. In the newborn mouse (Pl. 3, fig. 12) a definite lower sinus, with well-developed fibrous walls and connective tissue trabeculae, is found filled with blood cells. Three days later the lower sinus is larger (Pl. 3, fig. 13), and the upper sinus and Ringwulst are completely differentiated. The blood capillaries of the dermal papilla have already been formed in 17- and 18-day embryos (stage 8).

Medullation of vibrissae was observed only in adult mice, but the hairs are fully developed in all other respects at +3 days. At this stage the difference in size between vibrissae and pelage hairs has already begun to appear, and the keratinized vibrissae in the first few days after birth are about 12–18 μ in diameter, while those of pelage hairs are about 3–6 μ.

In some vibrissal follicles of the newborn mouse pyknotic nuclei were observed in the cells of the hair matrix and the outer root sheath. These changes may be the beginning of the normal process of shedding.

After birth no new vibrissal follicles are added to those already regularly arranged in rows. In the pelage, on the other hand, the new central primary follicles continue to appear until about +3 days, and in the next 5 days many new follicles develop around each of these to complete the adult arrangement in groups.

In the embryos the epidermis of the upper lip develops from a simple to a stratified epithelium as described for other body regions of the mouse by Hanson (1947), though at a slightly faster rate (Pl. 1, figs. 2, 4; Pl. 2, figs. 6, 7).

DEVELOPMENT OF VIBRISSAL FOLLICLES *IN VITRO*

(1) *Behaviour of skin in cultivation*

The explants of skin from the upper lip grew and differentiated in the same way as those of body skin (Hardy, 1949). During cultivation for periods of 5–14 days the explants gradually increased in volume, and there was an outgrowth of fibroblasts and usually also of epithelial cells. Many skin pieces from the lip began to curl with the epidermis inside, but unlike body skin they did not roll up completely in a ball. When a single row was explanted, the follicles usually grew out from the epidermis parallel to the clot surface (Pl. 3, fig. 15), but when a number of rows was included in the explant the follicles grew into the clot at right angles to its surface (Pl. 3, fig. 14). Whatever the orientation of the follicles with respect to the medium, their arrangement in rows was preserved during cultivation.

The simple embryonic epidermis of the upper lip, like that of the trunk (Hardy, 1949), differentiated in a normal manner *in vitro* (Pl. 3, figs. 15, 17; Pl. 4, fig. 20). The mesodermal cells beneath the embryonic epidermis became concentrated near the basement membrane as well as around the hair follicles, as they did *in vivo*. The formation of connective tissue fibres was occasionally observed in explants, but blood vessels did not develop.

(2) *Success in development of follicles*

In all the experiments there was some degree of follicle differentiation (Tables 2 and 3). Table 3 shows that in the Sydney series the development of vibrissal follicles in some explants was at a rate comparable with that in the embryo. The data for these and all subsequent tables were derived from both the living explants and the serial sections. Individual rates of development are discussed in more detail below. The numbers of explants in which some follicle differentiation took place, and the numbers in which hairs were formed, are recorded in Tables 4 and 5. In Table 5 the 'total number of explants' in the Sydney series include some which did not survive explantation and some which differentiated and then regressed; if these were omitted, the proportion of differentiating explants would be higher than 62 %. In this series 30 % of all explants showed hair development (stage 6), and this percentage is comparable with that for body skin explants (Hardy, 1949).

Table 2. *Summary of tissue-culture experiments of Galveston series*

Exp. no.	Age of embryo (days)	Initial stage	Final stage *in vitro*
2	11	0	3
3	14	3–4	8
1	15	5	8

Table 3. *Summary of tissue-culture experiments of Sydney series*

Exp. no.	Age of embryo (days)	Initial stage	Final stage *in vitro*	Minimum total age at final stage reached *in vitro* (days)	Normal age *in vivo* for final stage *in vitro* (days)
4	12	1	3	18	14
7	12	1	8	18	17–18
5	13	2	8	19	17–18
6	13	2	8	17	17–18

Table 4. *Numbers of explants in Galveston series showing progress in hair-follicle development*

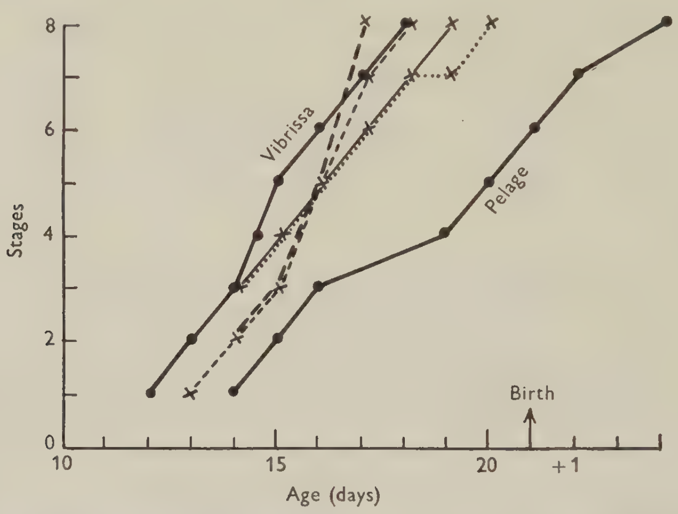
Exp. no.	Total no. of explants	No. of explants showing further follicle differentiation	Differentiated explants (%)	No. of explants differentiated to stage 6	Explants at stage 6 or later (%)
2	4	2	50	0	0
3	6	6	100	4	67
1	8	8	100	4	50
Grand total	18	16	89	8	44

Table 5. *Numbers of explants in Sydney series showing progress in hair-follicle development*

Exp. no.	Total no. of explants	No. of explants showing further follicle differentiation	Differentiated explants (%)	No. of explants differentiated to stage 6	Explants at stage 6 or later (%)
4	8	6	75	0	0
7	23	16	70	8	35
5	29	16	55	9	31
6	24	14	58	8	33
Grand total	84	52	62	25	30

(3) *Extent of development*

In cultivation many hair follicle rudiments developed from stage 1 or 2 to stage 8, in which a keratinized hair emerged from the skin (Tables 2 and 3). In the explants from 12-day embryos many individual follicle rudiments, which appeared only after cultivation had begun, underwent complete development from stage 0 to stage 8. In the intact animal hair growth continues for many days following the appearance of stage 8, a medulla is formed, and the dermal sheath acquires a system of blood sinuses and muscle attachments. In cultivation, the hairs frequently continued to grow for several days, but failed to develop a medulla, and there were no blood sinuses or muscles. Some follicles were quite healthy when fixed after 9–14 days of cultivation. In others the hairs had ceased to grow at about +1 day, and there were retrogressive changes in the lower parts of the follicles, including pyknosis in the hair matrix, although the other regions of the explants were healthy. Since these changes were similar to the retrogression observed in some follicles of a new-born mouse, they may merely represent the beginning of a normal process of shedding. In other explants in which the skin as well as the follicles became unhealthy, such an interpretation was not possible.



Text-fig. 4. Graph showing rates of development of vibrissal and pelage hair follicles (thick lines) and of individual vibrissal follicles in four explants (thin lines).

(4) *Rate of development*

It was noted from Table 3 that some explants reached their final stage of follicle differentiation in approximately the normal time. Text-fig. 4 shows that in a few the rate of development of follicles, stage by stage, was the same, within the experimental error, as that in the embryo. It will be recalled that the age of embryos was estimated only to the nearest day, and that explants were examined daily, so that recorded differences of one day in time of development have no significance. Differentiation in some explants was slower than in embryos, but since it was frequently impossible to see all the details of structure in the living state, many follicles must be wrongly designated in the records as slow in development.

The formation of hairs (stage 6), which can nearly always be recognized in living follicles, is a good criterion for the rate of development. Table 6 shows the mean age at which hairs were formed *in vitro* in each of three experiments. It will be noted that one-third of all the explants that reached stage 6 did so in the same time as if they had remained in the embryos.

Stage 8 is less easily recognized in living explants than stage 6, and it is therefore more likely that calculations based on the observation of stage 8 will err in showing slow development. Nevertheless, Table 7 shows that in three experiments at least one-quarter of the explants that reached this stage did so in the normal time.

The follicles which developed slowly *in vitro* were apparently not delayed at any one stage more than another. Vibrissal follicles *in vitro* had development curves resembling in shape the curve for normal vibrissal development, but differing from the curve for pelage hair follicles (Text-fig. 4).

Gradients, both in size of follicle and stage of development, were noted within each row of vibrissal follicles in the mouse. When a row of follicles was explanted, the same two gradients persisted throughout the period of cultivation.

Table 6. *Rates of development of hair follicles to Stage* 6 in vitro (16 *days* in vivo)

	Total explants which reached stage 6		Explants which reached stage 6 in shortest time	
Exp. no.	No.	Mean total age (days)	No.	Total age (days)
7	8	20·0	1	17
5	9	18·5	3	16
6	8	16·4	5	16

Table 7. *Rates of development of hair follicles to Stage* 8 in vitro (17–18 *days* in vivo)

	Total explants which reached stage 8		Explants which reached stage 8 in shortest time	
Exp. no.	No.	Mean total age (days)	No.	Total age (days)
7	4	20·3	1	18
5	6	20·0	1	19
6	2	17·0	2	17

(5) *Histology*

Vibrissal follicles *in vitro*, like pelage hair follicles, were completely normal in the histological structure of the dermal papilla, outer root sheath, Henle's layer, Huxley's layer, inner root sheath cuticle, hair cuticle and hair cortex. This fact is clearly seen by comparing Pl. 1, figs. 2–5 and Pl. 2, figs. 6, 7, with Pl. 3, figs. 15–17 and Pl. 4, figs. 18–21, in which the individual follicle layers can be recognized by the size, shape and internal structure of their cells (cf. also Maximow & Bloom, 1948). The hairs formed in explants were normal in structure and showed the typical serrated margin produced by the overlapping cuticle scales (Pl. 4, fig. 22). It will be recalled that in the above features no essential difference was found between the two follicle types *in vivo*. In addition, many structural features which distinguish vibrissal from pelage hair follicles appeared in explants of upper lip skin. The greater size of early vibrissal follicles and the hour-glass shape were noted. A many-layered outer root sheath developed the characteristic superior and inferior swellings (Pl. 4, fig. 19). A thick dermal sheath was present from the earliest stages (Pl. 3, fig. 17). The hair canal was formed in the same manner as in the vibrissal follicles *in vivo*.

The sizes of some vibrissae in explants were comparable with those in young mice of the same total age, and larger than pelage hairs. Those at 20 days figured in Pl. 4, figs. 20, 22, were 14 and 10 μ thick, and four hairs from an explant from a 13-day embryo after 12 days' cultivation were 9, 14, 15 and 17 μ thick respectively in the keratinized part.

In embryos there are only slight differences between vibrissal and pelage hair follicles in the rate at which sebaceous glands develop (p. 346). In explants of both upper lip and body skin the sebaceous gland swellings appeared, but so few gland cells differentiated that it was impossible to distinguish between the two regions with regard to sebaceous gland development in cultivated skin.

The other characteristics of vibrissal follicles which have been described, namely the special blood and nerve supply, and the intermediate type of medulla, did not develop *in vitro*. Blood vessels and nerves were not formed in any part of the explants.

DISCUSSION

The observations on the arrangement of vibrissae on the upper lip have merely confirmed those of other workers (Danforth, 1925*b*; Grüneberg, 1943*a*) except for minor differences in the numbers of follicles in some of the horizontal rows. However, the present study has extended the existing histological knowledge of adult mammalian vibrissal follicles (Szymonowicz, 1936) and made clear the distinction between these and pelage hair follicles in one species.

The inner root sheath collar, which closely invests the mouse vibrissa in its follicle below the sebaceous gland opening, is absent from the pelage hair follicles of the mouse (Dry, 1926), of the Australian opossum (Gibbs, 1938) and of the merino sheep (Duerden & Ritchie, 1924). This collar might therefore appear to be a distinctive feature of vibrissal follicles. However, a comparable structure has been described (Spencer & Sweet, 1899) in the follicles of spines in the Monotreme, *Tachyglossus aculeatus*. A more loosely fitting collar which is probably also a similar modification of the inner root sheath was noted in the follicles of coarse wool in some sheep (Burns & Auber, 1951) and in the follicles of coarse hairs in cattle (Auber, 1951). Since all three of the above follicle types belong to the pelage, it seems probable that the collar has no special significance in vibrissal follicles, but may exist for the mechanical support of any large fibres in their follicles.

In the development of follicles the most important qualitative difference between vibrissae and the pelage hairs is the manner of hair canal formation. The cap of epidermal cells which appears at the site of the canal at stage 5 in mouse vibrissal follicles is absent from the pelage hair follicles of the mouse, but has been noted in those of the guinea-pig (Segall, 1918). The formation of a canal by keratinization from the epidermal surface downwards distinguishes the vibrissal follicles of the mouse from the pelage hair follicles of this species (Hardy, 1949), of other Eutherians (Wildman, 1932) and of marsupials (Gibbs, 1938), in all of which the canal begins as a blind cavity within the deeper layers of the epidermis. In the guinea-pig (Segall, 1918) the pelage hair apparently emerges from the skin through a solid mass of epidermis. Poulton (1894) suggested that in the Monotreme, *Ornithorhynchus anatinus*, the whole of pelage hair formation took place within an epidermal tube open to the skin surface, but this was denied by Spencer & Sweet (1899), who found that the canal began to form only after the hair was about three-quarters of the way up the follicle. Although Spencer and Sweet's drawings suggest that keratinization of the canal in *Ornithorhynchus* began from the epidermal surface, the present description of canal formation in mouse vibrissal follicles is apparently the first one of such a method of development. In this study the sequence of events was confirmed by the examination of living follicles in tissue culture.

The theory that a hair canal is formed by the upward movement of cells derived from, or connected with, the sebaceous gland (Wildman, 1932; Gibbs, 1938, 1941) was not supported by the study of the pelage hair follicles of the mouse *in vivo* or *in vitro* (Hardy, 1949). The present observations on vibrissal follicles of the mouse show even more clearly that the sebaceous glands play no part in hair canal formation. In the mouse embryo the canals of vibrissae begin to form before the secretory sebaceous cells differentiate, and in explants the hair canals often completed their normal development in follicles which had no sebaceous cells.

Only the first two stages of development of vibrissal follicles in mammals have been described previously (Maurer, 1892; Zietzschmann, 1920). When the complete development was studied in the mouse it was found that many of the features which distinguish adult vibrissal from adult pelage hair follicles, such as size, shape and the thickness of the dermal sheath, appear or are fore-shadowed in the very early stages. The thicker outer root sheath of vibrissal follicles with superior and inferior swellings, the special type of hair canal and the thicker hair appear a little later. All these features developed in follicles from upper lip skin in tissue culture, while the opposite characteristics of pelage hair follicles developed in explants of skin from the trunk. The last of the vibrissal follicle characteristics to appear in the intact animal were found to be the intermediate type of medulla and the extra-epidermal blood and nerve supply and striated muscle fibres. These types of tissue did not develop at all in the explants, but most of them probably depend for their normal development on the maintenance of connexions with structures outside the small area of skin which is explanted. It may therefore be stated that all the main characteristics of vibrissal follicles which had an opportunity of expression in the tissue cultures did appear in explants from the upper lip. Thus the whole chain of development must be already determined in the original explants. It has already been pointed out (Hardy, 1949) that this effectively counters the suggestion by Danforth (1925*b*) that the differences between the two follicle types may be due to environmental influences acting on them during development.

The present study shows that upper lip skin can develop follicles and hairs when explanted at an earlier age than that previously reported (Hardy, 1951). For example, some follicle differentiation occurred in 11-day explants, and 12-day explants developed to stage 8, so that the capacity for differentiation of upper lip skin is comparable with that of trunk skin (Hardy, 1949). The fact that 11-day explants developed only to stage 3 may be due to limited potential but is more probably due to deficiencies of technique.

Perhaps the most interesting result from the tissue culture experiments is the accuracy with which the rate of vibrissal follicle development in some explants corresponded to the normal rate *in vivo*, and differed from that of pelage hair follicles. This shows that the relatively fast rate of vibrissal development in the embryo cannot be attributed to better circulation on the snout than on the trunk, or to any other local environmental factor, but is more probably an inherent property of the vibrissal follicle, just as its size and shape.

SUMMARY

1. The vibrissal follicles of adult mice differ from pelage hair follicles in many of their histological features.

2. Many characteristics which distinguish vibrissal from pelage hair follicles appear early in follicle differentiation.

3. During the development of the vibrissal follicle from embryonic skin the hair canal is formed by keratinization, which, unlike that of the pelage hair canal, begins at the surface of the epidermis.

4. Vibrissal follicles developed in 67 % of all explants of skin from the upper lip of 11- to 15-day mouse embryos cultivated by the hanging-drop method in a mixture of fowl plasma and chicken-embryo extract.

5. In some explants from 12-day and older embryos in which follicles were just beginning to form, keratinized hairs emerged from the skin after six or more days *in vitro*.

6. Vibrissal follicles developing *in vitro* showed many of those features which distinguish them *in vivo* from pelage hair follicles, including greater size, hour-glass shape, a thick dermal sheath and a special type of hair canal.

7. The rate of development of many vibrissal follicles *in vitro* was similar to that *in vivo*, and differed from the slower rate of development of pelage hair follicles.

8. The distinguishing features of vibrissae and pelage hairs which have been enumerated cannot be due to environmental influences during the development of follicles or hairs.

This work was commenced by one of us (M.H.H.) at the Tissue Culture Laboratory, University of Texas Medical Branch, Galveston, U.S.A., and aided by a grant from Hoffmann-La Roche, Inc. Gratitude is expressed to Prof. C. M. Pomerat who made this possible, and to all his staff at the Tissue Culture Laboratory for their helpful co-operation. The major part of the work was carried out as part of the research programme of the Division of Animal Health and Production, Commonwealth Scientific and Industrial Research Organization (Australia). Accommodation and facilities were kindly provided by Mr D. A. Gill at the F. D. McMaster Laboratory, Glebe, Sydney. The photographs were taken by Mr C. Poole.

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EXPLANATION OF PLATES

B	Basophilic nuclei of epidermal cap
C	Cortex (unkeratinized)
Co	Collar of inner root sheath
Cu	Hair cuticle
CuI	Inner root sheath cuticle
D	Dermal sheath
H	Hair
HC	Hair canal
He	Henle's layer
HN	Hair cone
Hx	Huxley's layer
IO	Inferior swelling of outer root sheath
K	Keratinized cells of hair canal
L	Lower blood sinus
O	Outer root sheath
P	Dermal papilla of hair follicle
PP	Pre-papilla
R	Ringwulst
S	Sebaceous gland swelling
SG	Sebaceous gland with differentiated cells
SO	Superior swelling of outer root sheath
1	Stage 1
2	Stage 2

All figures are of sections through vibrissal follicles in skin of the upper lip from embryonic or post-natal mice directly or after culture. The staining was with Mayer's haemalum, eosin and picric acid.

Plate 1

Fig. 1. Vertical section of upper part of active follicle of adult. × 150.

Fig. 2. Vertical section of skin of 14-day embryo, with two follicles at stage 1, and one at stage 2. × 250.

Fig. 3. Vertical section of follicle of 14-day embryo, at stage 3. × 250.

Fig. 4. Vertical section of follicle of 15-day embryo, at stage 4. The epidermis shows a stratum spinosum. × 250.

Fig. 5. Vertical section of follicle of 15-day embryo, at stage 5. × 250.

PLATE 2

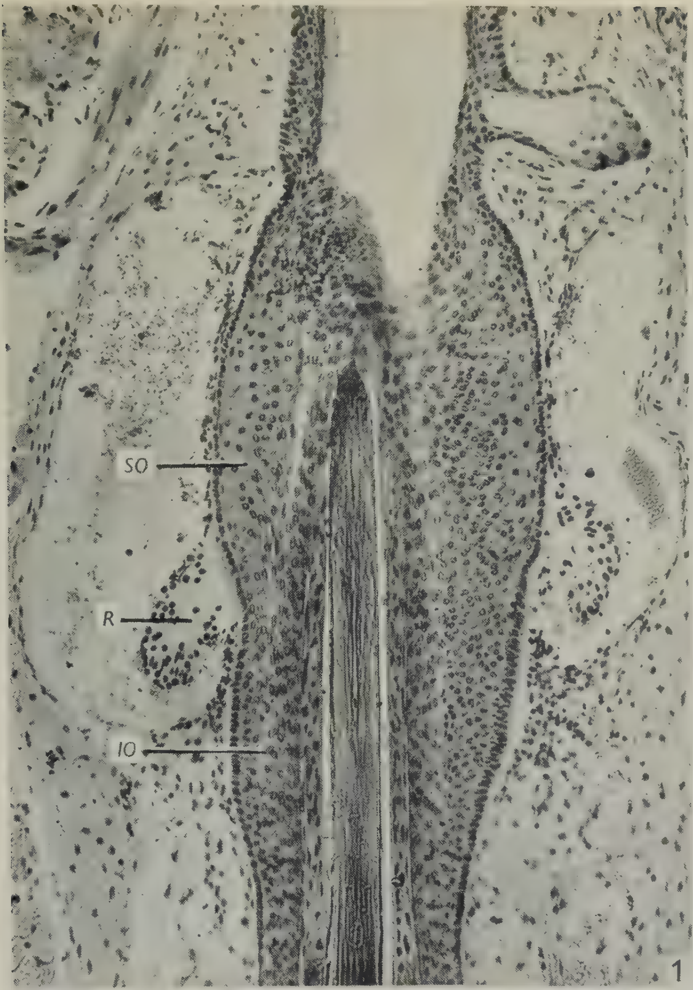
Fig. 6. Vertical section of follicle of 16-day embryo, at stage 6*a*. The epidermis has a stratum granulosum. ×250.
Fig. 7. Vertical section of follicle of 17-day embryo, at stage 8. The epidermis shows a stratum corneum. ×150.
Fig. 8. Hair canal of follicle at stage 5, from 15-day embryo. ×400.
Fig. 9. Hair canal of follicle shown in fig. 6, at higher magnification. ×400.
Fig. 10. Hair canal of follicle at stage 6*b*, from 16-day embryo. ×400.
Fig. 11. Hair canal of follicle at stage 7, from 17-day embryo. The collar formed by the inner root sheath is visible below the canal. ×400.

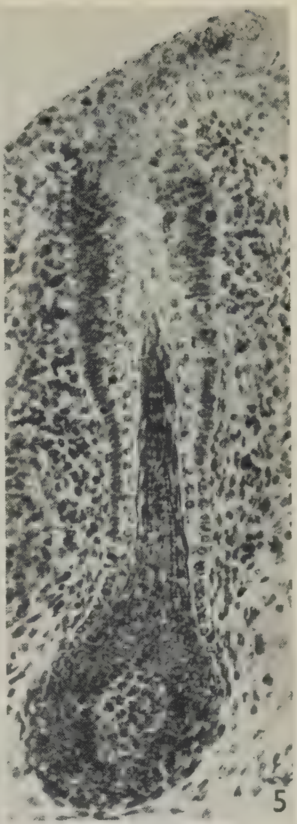
PLATE 3

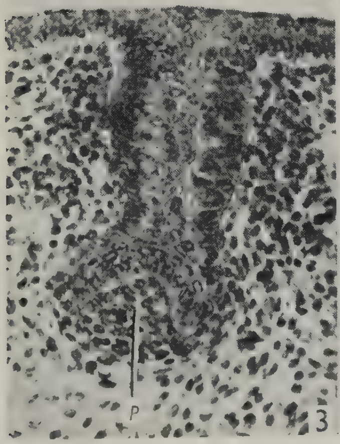
Fig. 12. Oblique section of follicle from newborn mouse. ×150.
Fig. 13. Vertical section of follicle from 3-day-old mouse. ×150.
Fig. 14. Horizontal section of entire explant of skin from 15-day embryo, after 6 days *in vitro*, showing development of follicles in rows (Exp. 2). ×100.
Fig. 15. Part of explant of skin from 12-day embryo, after 8 days *in vitro*, showing two follicles at stage 2 (Exp. 7). ×250.
Fig. 16. Vertical section of follicle at stage 3, in explant of skin from 12-day embryo after 8 days *in vitro* (Exp. 7). ×250.
Fig. 17. Part of explant of skin from 13-day embryo, after 9 days *in vitro*, showing follicle on right at stage 4, and follicle on left at stage 5 (Exp. 5). ×250.

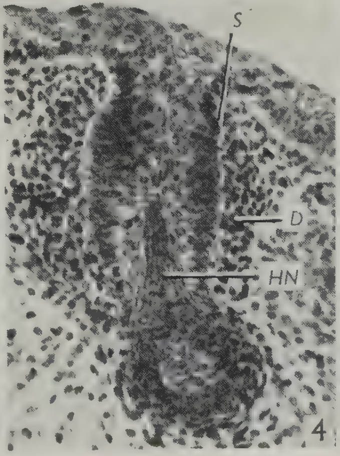
PLATE 4

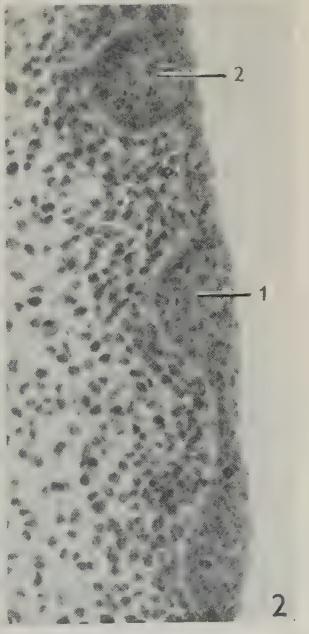
Fig. 18. Base of follicle at stage 5, in explant of skin from 12-day embryo, after 4 days *in vitro*, showing follicle layers (Exp. 7). ×250.
Fig. 19. Follicle at stage 6 from explant shown in fig. 14. ×250.
Fig. 20. Another area of explant shown in fig. 16, with one follicle at stage 8, and another, cut obliquely, at stage 7. The epidermis is fully differentiated. ×150.
Fig. 21. Section parallel to that shown in fig. 20, showing cell layers at the base of follicle at stage 8. ×250.
Fig. 22. Section adjacent to that shown in fig. 20, showing cuticular scale pattern on surface of hair in follicle at stage 7. ×1800.



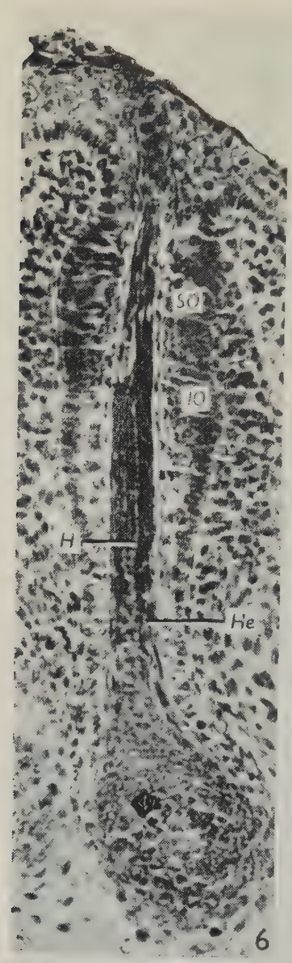


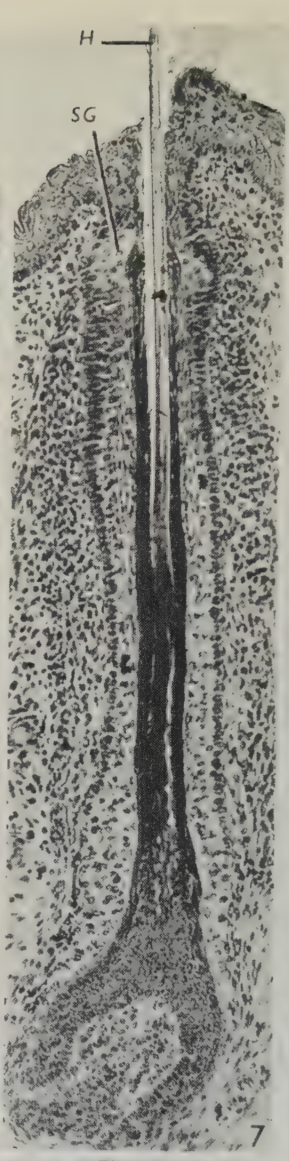


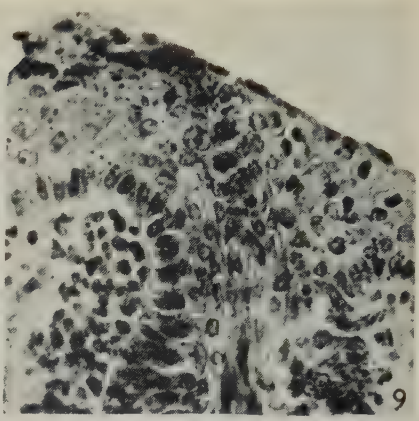


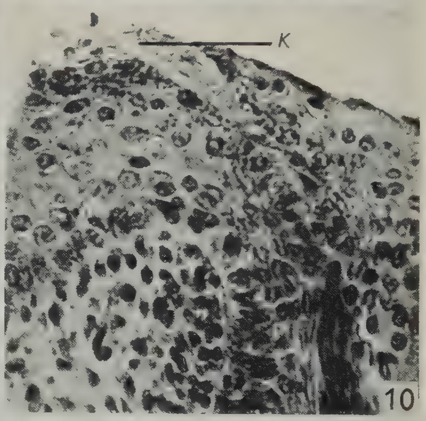


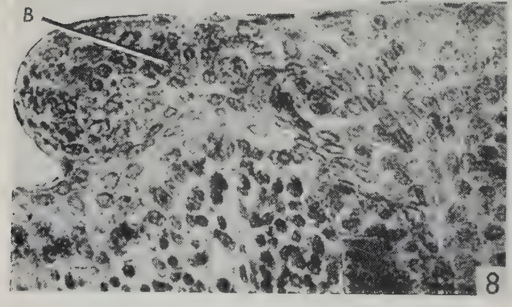
DAVIDSON AND HARDY—THE DEVELOPMENT OF MOUSE VIBRISSAE *IN VIVO* AND *IN VITRO*

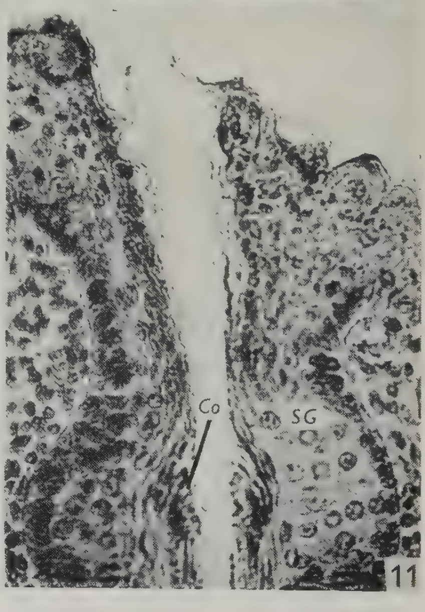












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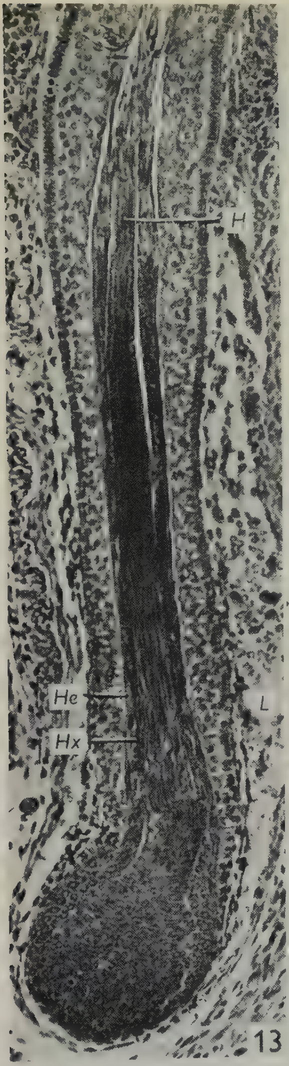

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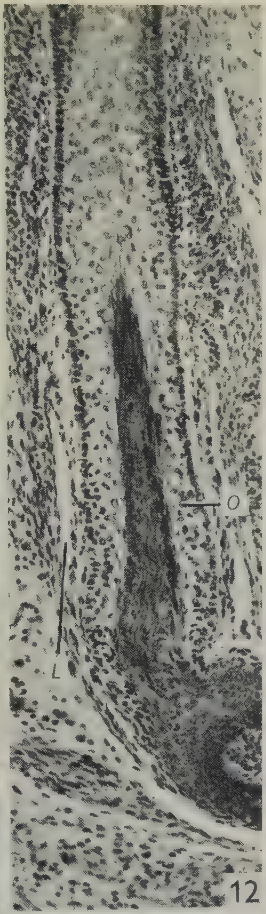

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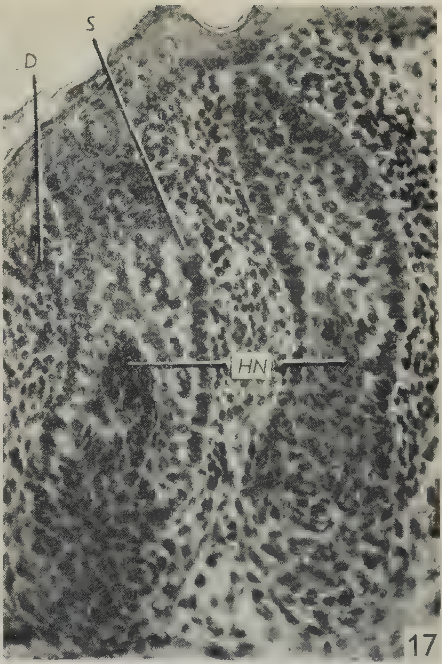

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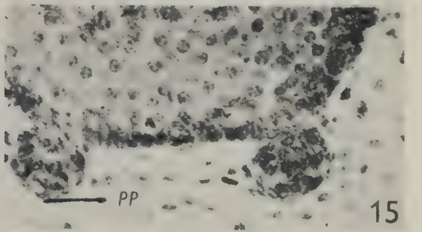

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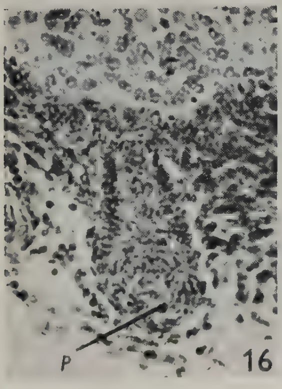

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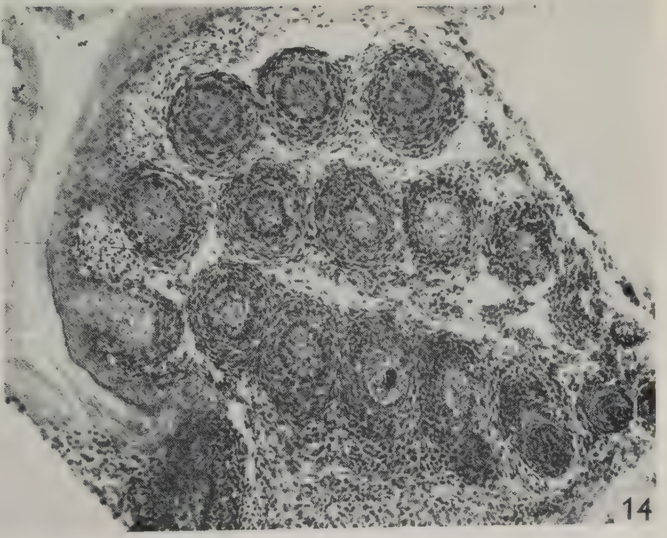

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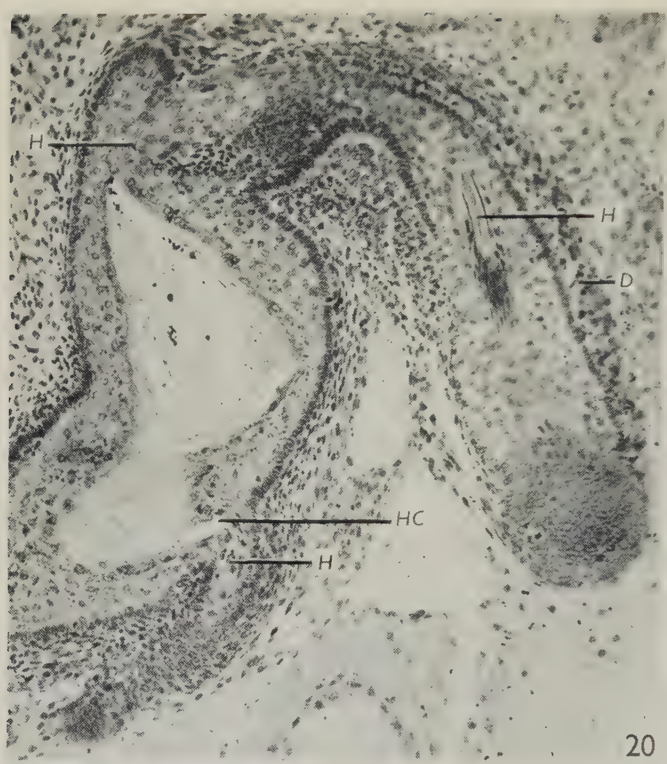

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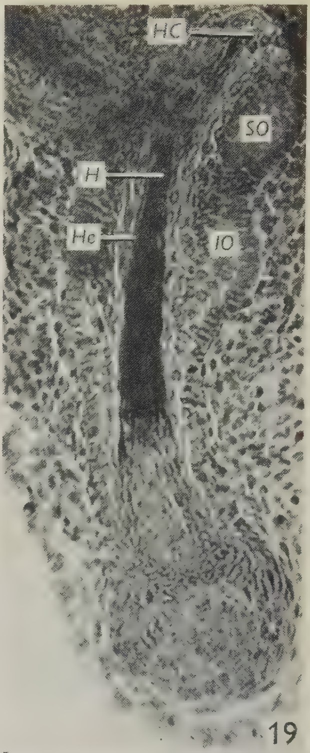

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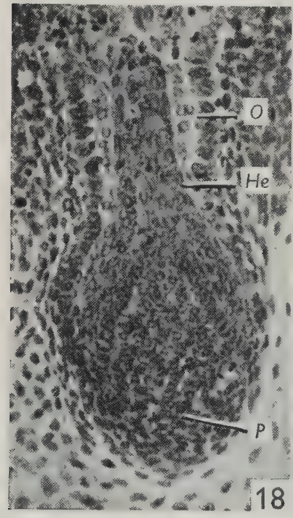

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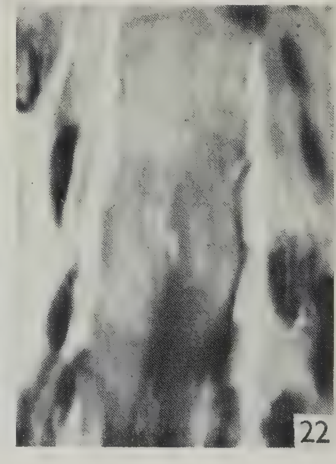

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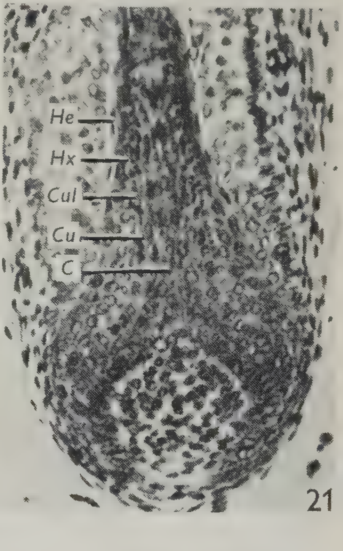

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DAVIDSON AND HARDY—THE DEVELOPMENT OF MOUSE VIBRISSAE *IN VIVO* AND *IN VITRO*

THE PRENATAL DEVELOPMENT OF THE ABDOMINAL PARA-AORTIC BODIES IN MAN

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INTRODUCTION

The adrenaline secreting cells of the human foetus are found in two situations, viz. in the adrenal glands and associated with the sympathetic nervous system outside these structures. These cells are usually demonstrated histologically by making use of the chromaffin reaction and are known as chromaffin cells or phaeochromocytes.

In the abdomen of the foetus the extra-adrenal phaeochromocytes are found either in small non-encapsulated groups in sympathetic ganglia, or in encapsulated masses in the prevertebral sympathetic plexuses, the latter being the more common arrangement. The encapsulated collections of cells are usually referred to as 'paraganglia', but in this work (for reasons which will be discussed later) the latter term has been replaced by 'para-aortic bodies'.

HISTORICAL SURVEY

Stilling (1890) observed extra-adrenal chromaffin cells lying in the midst of the abdominal sympathetic plexuses of the rabbit, cat and dog.

Zuckerkandl (1901) described extra-adrenal chromaffin cells in the abdominal sympathetic plexuses of the human foetus and described in detail collections of these cells closely related to the origin of the inferior mesenteric artery. These are now usually referred to as the 'organs of Zuckerkandl'.

Bonnamour & Pinatelle (1902) confirmed Zuckerkandl's findings and described chromaffin bodies in children up to the age of 6 years. Kohn (1902) reviewed the literature relating to the chromaffin bodies of vertebrates, and in 1903 published his classical paper on the development of the paraganglia in man, rabbit and cat; in the latter paper the development of chromaffin cells from the sympathetic anlage was clearly described.

Vincent (1910) described the adrenal and extra-adrenal chromaffin tissues of the dog, cat and rabbit, and showed that in the dog the extra-adrenal tissue contained a pressor substance.

Zuckerkandl (1912) reviewed the literature relating to chromaffin tissues, and made contributions in the field of the postnatal fate of these structures.

From 1925 onward, an important series of papers was published, all relating to the development of the para-aortic bodies in man. Ivanoff (1925) described the development and postnatal degeneration of the 'paraganglia'. Wrete (1927) observed the continuity of the chromaffin bodies of the para-aortic region with the chromaffin tissue of the adrenal glands. Keene & Hewer (1927) observed the common origin of the intra- and extra-adrenal chromaffin cells from the sympathetic anlage. Iwanow (1930, 1932) described in detail the morphology and embryology of the 'paraganglia' in man.

The descriptive works of Kohn (1903) and later workers established the common origin of the cells of the adrenal medulla and para-aortic bodies from the sympathetic anlage. This concept was supported experimentally by the work of Van Campenhout (1930) on amphibians, and Hammond & Yntema (1947) on chicks.

Hollingshead (1937) showed that extra-adrenal chromaffin tissue was innervated by pre-ganglionic sympathetic nerve fibres, and in 1940 reviewed the literature relating to these structures. Van Campenhout (1946) included 'paraganglia' under the heading 'epithelio-neural bodies'.

It has recently been shown (West, Shepherd & Hunter, 1951) that the pressor substance present in the organs of Zuckerkandl of the newborn infant is predominantly noradrenaline.

NOMENCLATURE

Kohn (1900, 1902, 1903) referred to the extra-adrenal collections of chromaffin cells as 'paraganglia'. Within this term he included those structures which were developed from the sympathetic anlage, which were topographically related to the sympathetic nervous system and which were composed of chromaffin cells; the carotid body was included, and he wrote (1902): 'Die carotisdrüse ist ein chromaffiner Körper oder ein Paraganglia und richtiger als Paraganglion caroticum zu bezeichnen'. Kohn's inclusion of the carotid body amongst the 'paraganglia' has not, however, been confirmed by subsequent workers (Mönkeberg, 1905; De Castro, 1926, 1928; Boyd, 1937; Gosses, 1937). There is general agreement that neither developmentally (Boyd, 1937) nor functionally (Christie, 1933; Gosses, 1937) does this structure justify inclusion amongst the chromaffin organs.

The term 'paraganglion' may, therefore, be applied in either a specific sense as implied by Kohn (1900, 1902, 1903) and used by Boyd (1937) and Hammond (1941) to describe chromaffin structures developed from and topographically related to the sympathetic nervous system—the carotid body being excluded for the reasons given above—or non-specifically as used by Goormaghtigh (1936). The latter author extended the term further by including 'vagal paraganglia' of the mouse and recognized three types of paraganglia, viz. orthosympathetic, parasympathetic (vagal) and sensory (carotid body).

Because of the lack of uniformity in the interpretation of the term 'paraganglia', it has been replaced in the present investigation by the topographical term 'para-aortic bodies'. A para-aortic body is defined as an encapsulated collection of chromaffin cells lying in intimate contact with the sympathetic nervous system and yielding a pressor substance after extraction.

THE CHROMAFFIN REACTION

Henle (1865) observed a brown coloration in the cells of the adrenal medulla after treatment with chromic acid or chrome salts. This reaction was referred to by Stilling (1890) as the 'chromaphil' reaction and by Kohn (1900) as the 'chromaffin' reaction.

Ogata & Ogata (1923) reviewed the literature relating to the chromaffin reaction, and, after comparing the changes produced by the action of potassium dichromate on solutions of adrenaline and on cells of the adrenal medulla, concluded that the brown coloration was due to the production of chromium compounds in the affected cells.

Gerard, Cordier & Lison (1930) considered that the reaction was independent of the presence of chrome compounds and was due to the oxidation of adrenaline and related substances, with the formation of quinones and brown tar-like derivatives. This work was confirmed by Bennett (1941) who suggested that the term 'chromaffin reaction' should be replaced by 'fuscogenic reaction'. Van Campenhout (1946) suggested that 'reaction of Henle' would be a preferable term.

In the present investigation the older term 'chromaffin reaction' is retained as being one in general use and most suitable, and refers to the brown coloration produced by the action of potassium dichromate upon cells which contain a pressor substance (adrenaline or noradrenaline).

MATERIAL AND METHODS

Eighteen human foetuses and five newborn infants were used. The sizes (in mm.) of the former were: 8, 12, 14 (two), 16 (two), 19·5, 27, 46, 52, 55, 84, 92, 108, 135, 142, 150 and 270.

Only very fresh material was used; this was obtained whenever possible after hysterotomy or after medical induction of abortion. All specimens of 16 mm. and less were fixed in Bouin or formol-saline, the 52 mm. foetus was fixed in Bouin and the 150 mm. foetus in Zenker. All other foetal material was fixed in formol-dichromate (neutral formaldehyde 5 %, potassium dichromate 3 %). Fixation was accomplished by injection and immersion. The larger foetuses were eviscerated prior to immersion. All specimens between 8 and 19·5 mm. were sectioned serially at 8–10 μ, as were the posterior abdominal walls of foetuses of 27, 52, 92, 108, 142, 150 and 270 mm. The other foetuses were examined macroscopically after treatment by the Wislocki method (1922) which bleaches all structures except those giving a positive chromaffin reaction.

The five infants all died within 1 day of birth, three were premature weighing 3lb. 6oz. and 3lb. 10oz. (two); in these pregnancy had reached the 32nd week. The others weighed 7 lb. and 7 lb. 6 oz. and pregnancy had reached full term. From these five infants the posterior abdominal viscera were excised *en masse* and the tissue immersed for 3 days in formol-dichromate. The posterior abdominal wall of one 3 lb. 10 oz. infant was sectioned serially at 12 μ; the others were examined macroscopically after treatment by the Wislocki method.

Sections were stained either with haematoxylin and eosin, or by Masson trichrome (iron haematoxylin, Ponceau acid-fuchsin, aniline blue) or Giemsa methods for special purposes.

OBSERVATIONS

8 *mm. stage*

At this stage the sympathetic anlage forms a chain along the dorso-lateral aspect of the abdominal aorta (Pl. 1, fig. 1). A few primitive sympathetic cells (sympathogonia) may be observed extending ventrally on both sides of the abdominal aorta but a pre-aortic plexus is not observed.

In sections cut at and just cranial to the origin of the coeliac artery, the rudiments of the epithelial adrenal glands may be observed, closely related to columnar cells, which line the coelom on each side of the mesentery.

12 *mm. stage*

The sympathetic anlage has extended into the pre-aortic region and a definite pre-aortic plexus exists. The epithelial adrenals have increased in size and on their medial aspects are closely related to sympathetic elements—primitive cells and fibres. The sympathetic chains lie relatively nearer the vertebral bodies and farther from the aorta than at the 8 mm. stage.

14 *mm. stage*

Sections at the level of the adrenal glands show the early invasion of these structures by nerve fibres and primitive sympathetic cells (Pl. 1, fig. 2).

The pre-aortic plexus is well defined (Pl. 1, fig. 3), but there is no evidence of cellular differentiation within the sympathetic anlage in any area.

The primitive cells are characterized by small intensely basophilic nuclei (4–6 μ) in which scattered granules of chromatin are just visible; the cytoplasm is scanty.

16 *mm. stage*

At 16 mm. the invasion of the adrenal glands by sympathetic elements is more advanced and some of the cells on the medial aspects of these structures, and in the pre-aortic plexus, are arranged in characteristic whorls (Pl. I, fig. 4). The more central cells within the whorls have moderately basophilic ovoid nuclei, 6–8 μ in diameter, in which a few chromatin granules may be observed; the amount of cytoplasm is greater than in the primitive cells. These cells are phaeochromoblasts. Primitive cells persist in the peripheral zones of the whorls.

19·5 *mm. stage*

The whorls of phaeochromoblasts are larger and more numerous than in embryos of 16 mm. The largest are situated on each side of the mid-line in the pre-aortic region (Text-fig. 1, Pl. 2, fig. 6) and are related to the origin of the inferior mesenteric artery; smaller structures are found in other parts of the pre-aortic plexus (Pl. 2, fig. 5), along the medial aspects of the adrenal glands and kidneys and extend into the pelvis.

Primitive cells may be observed in the peripheral zones of the whorls and frequently exhibit mitosis; they are, however, relatively less numerous than in the 16 mm. stage. Occasional mitotic figures are seen in the more central phaeochromoblasts. The structures are poorly vascularized. Nerve fibres, primitive sympathetic cells and a few phaeochromoblasts can be traced into the medial aspects of the adrenal glands (Plate 3, fig. 7).

27 *mm. stage*

The arrangement of phaeochromoblasts is as in the previous stages. The cell whorls have increased in size, vascularization is taking place, but there is no evidence of further differentiation.

46–55 mm. stage

Between the 27 and 46 mm. stages, the majority of the phaeochromoblasts of the cell whorls and adrenal glands undergo further differentiation.

The change is again characterized by a decrease in nuclear basophilia—this results in the chromatin granules and threads becoming more obvious, and, together with a small nucleolus, they impart a 'dusty' appearance to the nucleus. The nuclei are round or ovoid and approximately the same size (6–8 μ) as those of the phaeochromoblasts. Associated with the nuclear change there is an increase in the amount of cytoplasm and the cells become rearranged with the formation of cords.

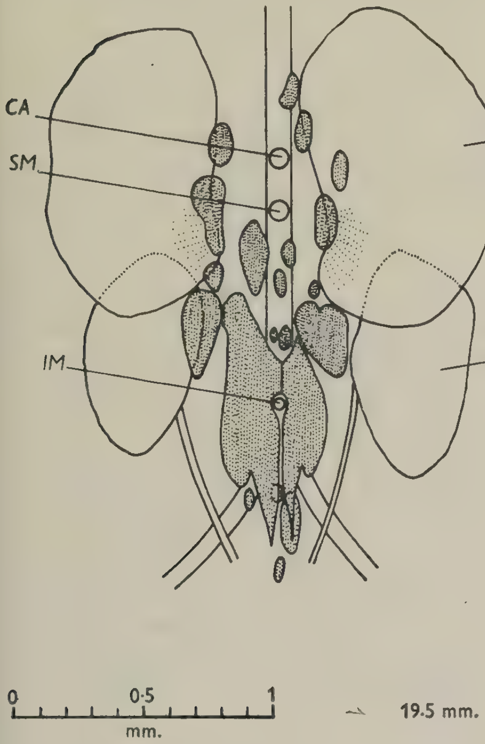


Fig. 1.

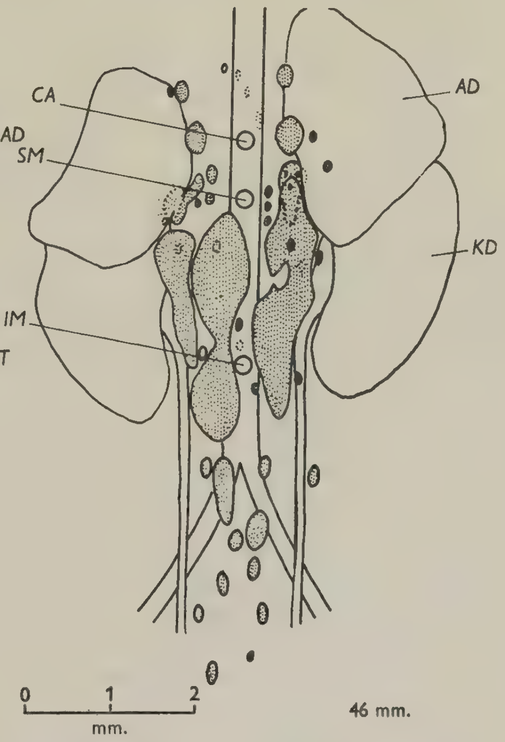


Fig. 2.

Text-fig. 1. Reconstruction of a 19·5 mm. human foetus, whorls of phaeochromoblasts stippled. *AD*, adrenal gland; *CA*, coeliac axis; *SM*, superior mesenteric artery; *IM*, inferior mesenteric artery; *MT*, metanephros.

Text-fig. 2. Reconstruction of a 46 mm. human foetus showing the distribution of the para-aortic bodies. Phaeochromocytes are extending from a para-aortic body into the right adrenal gland. *KD*, kidney. Other abbreviations as in Text-fig. 1.

A positive chromaffin reaction is not observed in microscopic sections at this stage, but in all other respects the cells are identical with those of older foetuses which do give a positive reaction, and are regarded as being phaeochromocytes. A positive reaction was observed in a gross specimen of 55 mm.

Associated with the cellular differentiation there is a marked increase in the vascularity of the structures, and networks of sinusoidal capillaries may be observed. The cords of phaeochromocytes and associated network of capillary blood vessels are surrounded by a thin capsule (Pl. 3, fig. 8). From this stage onwards the term para-aortic body is used to describe these structures.

In sections through the adrenal glands of 46 and 52 mm. foetuses, phaeochromocytes are observed extending from cranial para-aortic bodies into the adrenal glands (Text-figs. 2, 3; Pl. 4, fig. 9).

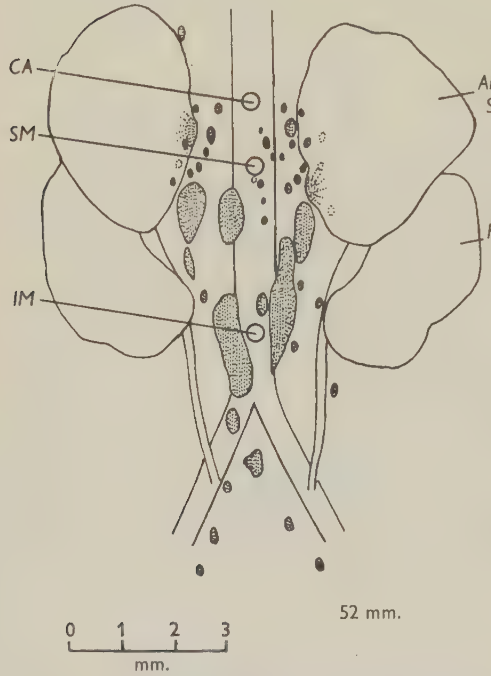


Fig. 3.

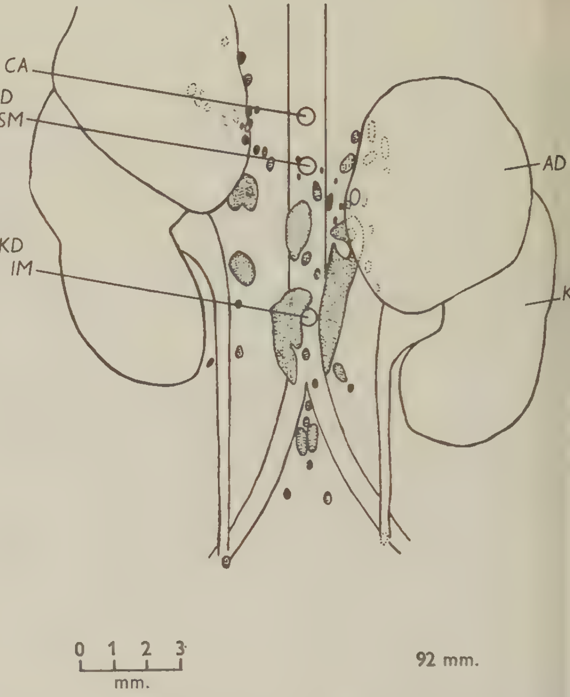


Fig. 4.

Text-fig. 3. Reconstruction of a 52 mm. human foetus. Para-aortic bodies stippled. Phaeochromocytes are extending from two cranial para-aortic bodies into the adrenal glands.

Text-fig. 4. Reconstruction of a 92 mm. human foetus. Para-aortic bodies stippled.

Large numbers of primitive sympathetic cells may be seen in the sympathetic chains and in the prevertebral plexuses, and are commonly arranged in rosette fashion. They are also present in the peripheral zones of all para-aortic bodies (Pl. 3, fig. 8).

The first sign of differentiation of primitive cells into sympathoblasts is noted at 46 mm., and occurs in the pre-vertebral plexus around the origin of the superior mesenteric artery. This differentiation is also characterized by a decrease in nuclear basophilia and the early cells are individually indistinguishable from phaeochromoblasts.

The arrangement of para-aortic bodies at 46 and 52 mm. (Text-figs. 2 and 3) corresponds in general with the cell whorls of the 19·5 mm. embryo. They extend in a cranio-caudal direction from the diaphragm to the pelvis and laterally as far as the uro-genital structures. They can be divided into:

(1) A median group of structures closely related to the aorta and in the mid-line of the pelvis.

(2) and (3) Two lateral groups which are in close proximity to the adrenal glands, kidneys, ureters and lateral pelvic walls.

Each of these groups is further divisible into:

(*a*) Large bodies which correspond in position with the cell whorls of the 19·5 mm. embryo and which in larger foetuses are macroscopic.

(*b*) Small bodies which do not correspond with the cell whorls of the 19·5 mm. embryo and which are always microscopic. The small bodies in the inter-adrenal area are usually deeply embedded in the sympathetic plexuses.

The largest bodies are members of the median group, and are in relation to the origin of the inferior mesenteric artery—these are the bodies commonly called the organs of Zuckerkandl.

Para-aortic bodies are associated with the aortic, adrenal, renal and testicular or ovarian plexuses. They are also present in the pelvis near the midline posteriorly, and lying lateral to the rectum, associated with the extensions of the pelvic sympathetic plexus. Bodies are not seen in relation to the gangliated sympathetic chains at this stage.

84–92 *mm. stage*

The para-aortic bodies increase in size as the foetus grows. Primitive cells are still present at the periphery of the bodies but are less numerous than in the earlier stages.

The primitive cells lying in the ganglia of the sympathetic chains have begun to differentiate, but chromaffin cells are not observed in these ganglia at this stage.

Phaeochromocytes of the adrenal glands and para-aortic bodies give a positive chromaffin reaction. Primitive cells can still be traced along nerve fibres into the adrenal glands, and form well-defined clusters and an occasional rosette in the interior of the glands.

Bodies exist in the same sites as in the 52 mm. foetus, i.e. in all parts of the prevertebral plexuses (Text-fig. 4). There is a slight increase in number of the small bodies between and posterior to the adrenal glands.

108–150 *mm. stage*

The general distribution of para-aortic bodies (Text-figs. 5–7) is the same as in the earlier stages. There is a gradual increase in the size of the large bodies and in the number of the small ones. As the former increase in size the connective tissue stroma becomes obvious. In foetuses of 108 and 150 mm. para-aortic bodies may be seen lying in the centre of developing lymph nodes (Pl. 4, fig. 10). The position of these bodies is indicated by arrows in Text-figs. 5 and 7.

In the same foetuses and in a 142 mm. foetus nerve fibres were observed traversing lymph nodes.

In foetuses of 142 and 150 mm. the organs of Zuckerkandl are united by an isthmus (Text-figs. 6 and 7), and in the larger foetus have a maximum length of 6 mm. The largest para-aortic body of the lateral groups is 2·8 mm. long.

In a 108 mm. foetus a small non-encapsulated collection of chromaffin cells is observed in a ganglion of the sympathetic chain.

Primitive sympathetic cells exist in the peripheral zones of all para-aortic bodies but become less numerous as the age of the foetus increases.

270 *mm. to full term*

A foetus of 270 mm. was sectioned after removal of kidneys and right adrenal gland. During this manipulation a number of the bodies belonging to the lateral groups was inadvertently removed, and this fact explains their absence in Text-fig. 8.

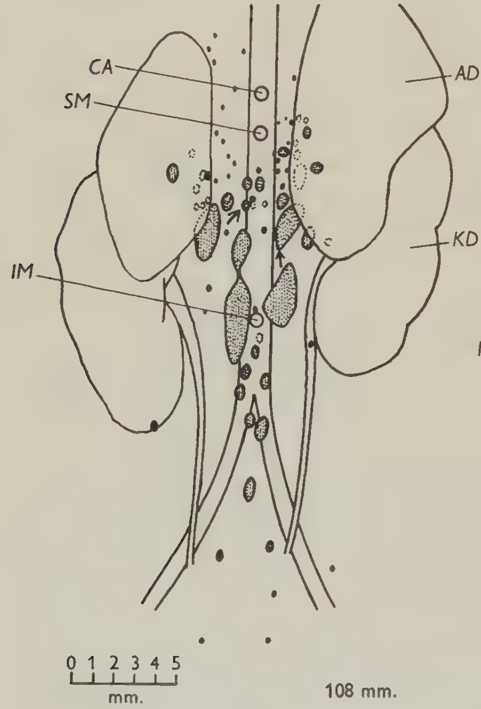


Fig. 5.

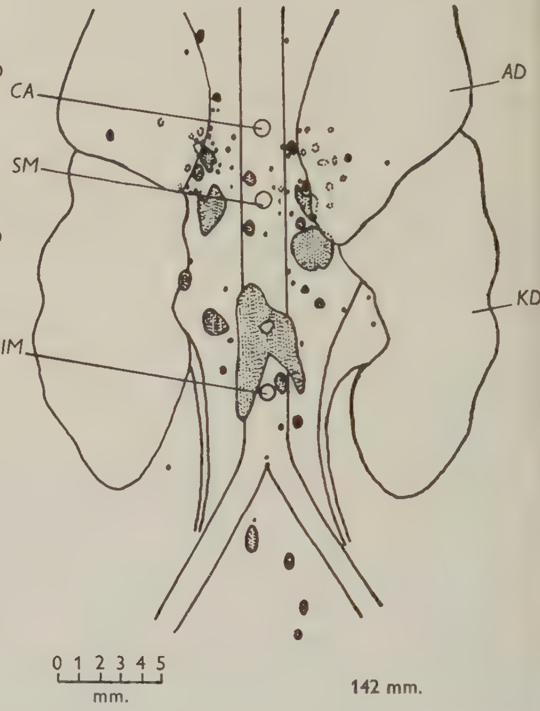


Fig. 6.

Text-fig. 5. Reconstruction of a 108 mm. human foetus. Para-aortic bodies stippled. The arrows indicate sites of close association between chromaffin and lymphoid tissue.

Text-fig. 6. Reconstruction of a 142 mm. human foetus. Para-aortic bodies stippled. The organs of Zuckerkandl are connected by an isthmus.

Primitive sympathetic cells are not observed in the peripheral zones of para-aortic bodies during this stage, and mitotic figures are absent.

In a 270 mm. foetus (Text-fig. 8) the majority of the pre-aortic chromaffin tissue is arranged as an elongated body 16 mm. long, extending from just caudal to the origin of the superior mesenteric artery to the origin of the inferior mesenteric artery. Bodies of 1–1·5 mm. in length exist near the midline of the pelvis and are associated with the fibres of the pelvic plexus; similar structures lie along the lateral extensions of this plexus and lie between the rectum and lateral pelvic walls. In two 3 lb. 10 oz.

premature infants (Text-fig. 9), the organs of Zuckerkandl are united by isthmuses and have a maximum length of 5 and 8 mm.; the larger bodies of the lateral groups are 2 mm. long. The total amount of extra-adrenal chromaffin tissue present in a 3 lb. 10 oz. infant (Text-fig. 9) appears to be approximately the same as that present in a 150 mm. foetus (Text-fig. 7).

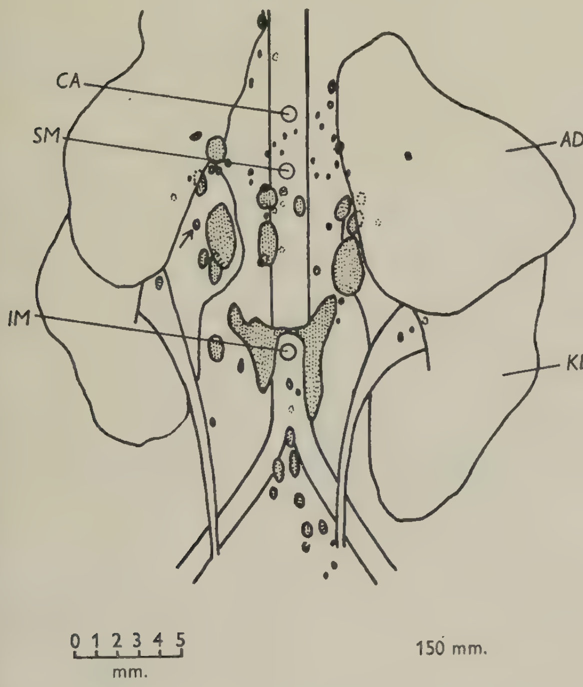


Fig. 7.

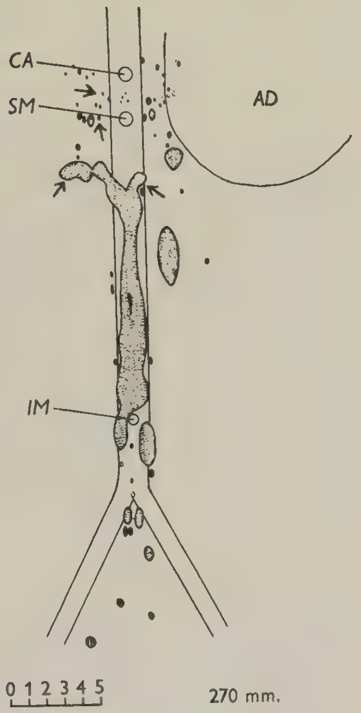


Fig. 8.

Text-fig. 7. Reconstruction of a 150 mm. human foetus. Para-aortic bodies stippled. The arrow indicates a body which is closely related to lymphoid tissue. The organs of Zuckerkandl are connected by an isthmus.

Text-fig. 8. Reconstruction of a 270 mm. human foetus, showing the more medially placed para-aortic bodies (stippled). The arrows indicate sites of close association between chromaffin and lymphoid tissue.

In a 7 lb. one-day-old infant the organs of Zuckerkandl measure 10 mm. in length and are unconnected; in a 7 lb. 6 oz. infant the structures are united by an isthmus and have a maximum length of 11 mm.

Primitive cells still form a prominent feature of the adrenal medulla and may be traced along nerve fibres from the periphery of the cortex to the interior of the glands in a 270 mm. foetus.

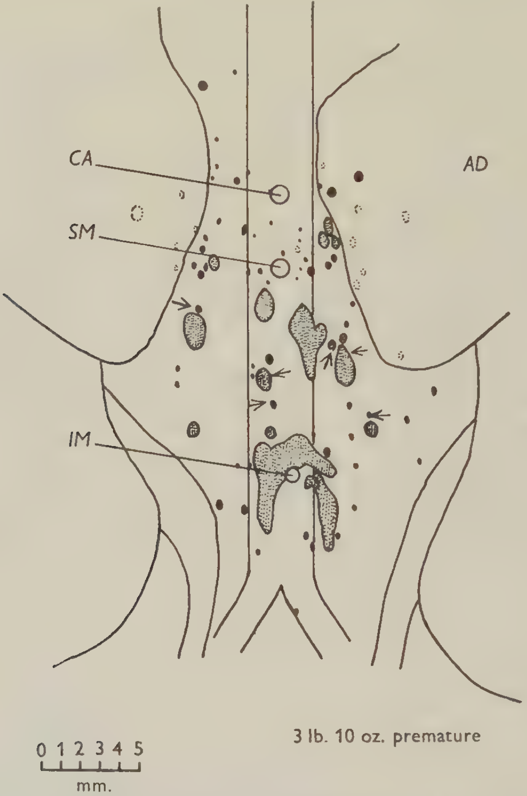
Non-encapsulated collections of phaeochromocytes are observed in the ganglia of the prevertebral plexuses and the sympathetic chains of a 270 mm. foetus and a 3 lb. 10 oz. premature infant. A small para-aortic body is also found associated with a ganglion of the sympathetic chain in the former specimen.

The close association between lymphoid tissue and chromaffin tissue is more widespread at this stage than in the preceding one; the sites of such association are indicated by arrows in Text-figs. 8 and 9.

This association takes two forms:

(1) Para-aortic bodies are seen lying in the centre of lymph nodes.

(2) The two structures lie side by side in contact with each other.



Text-fig. 9. Reconstruction of the posterior abdominal wall of a 3 lb. 10 oz. premature infant. Para-aortic bodies stippled. Arrows indicate sites of close association between chromaffin and lymphoid tissue. The organs of Zuckerkandl are connected by an isthmus.

At the points of contact, the capsules of the para-aortic bodies are incomplete, and lymphocytes may be observed infiltrating the chromaffin tissue by direct extension (Pl. 4, fig. 11). Perivascular collections of lymphocytes are also occasionally observed.

The larger bodies have a well-developed connective tissue stroma and a rich blood supply; the phaeochromocytes are arranged in cords between capillary blood vessels.

In gross specimens the para-aortic bodies are intimately connected with the nerve fibres of the prevertebral plexuses. In unfixed material they have a pinkish colour which is slightly different from the grey of the nearby lymph nodes, but they can only be identified with certainty after fixation in formol-dichromate and bleaching by the Wislocki method.

THE BLOOD SUPPLY OF THE PARA-AORTIC BODIES

In general, it may be said that arterial blood is derived from the nearest artery and the venous drainage is into a neighbouring vein. Branches of the aorta, superior mesenteric, inferior mesenteric and testicular or ovarian arteries have been traced to bodies in the older foetuses. In a 46 mm. foetus the large para-aortic bodies are supplied by branches of the aorta, the mesenteric arteries or the lateral splanchnic arteries (Pl. 3, fig. 8). These findings are in accord with the observations of Zuckerkandl (1901) and Iwanow (1932).

THE CHROMAFFIN REACTION

A positive chromaffin reaction was first observed in a gross specimen of 55 mm. after fixation in formol-dichromate and bleaching by the Wislocki method. In microscopic sections a reaction was first observed in the adrenal glands and para-aortic bodies of a 92 mm. foetus and was noted in all larger specimens. The intensity of the brown coloration varied from cell to cell. The lack of uniformity in the coloration of the phaeochromocytes may be due to the cells being in different phases of a secretory cycle, as described by Bennett (1941).

The presence of a pressor principle in the para-aortic bodies and adrenal glands of four foetuses between the sizes of 70 and 107 mm. was demonstrated by injecting extracts of these structures—obtained by the method of Holton (1949)—intravenously into chloralosed cats, and making simultaneous recordings of changes in the blood pressure and contractions of the nictitating membrane.

DISCUSSION

It is generally accepted that the chromaffin cells and sympathetic neurones are derived from the sympathetic anlage. The earlier work relating to the development of the chromaffin system (Kohn, 1903; Zuckerkandl, 1912) is characterized by a lack of uniformity in nomenclature relating to the various stages in the differentiation of the primitive cells. Kohn (1903) described ‘chromaffinen Zellen’ in a 19·5 mm. embryo and reported their subsequent change in size and staining reactions as further differentiation occurred, but retained the original name. Zuckerkandl (1912) referred to the primitive cells as ‘sympathoblasts’ or ‘chromaffinoblasts’, and all subsequent derivatives of the latter cells as ‘chromaffin bodies’.

In this paper the small basophilic cells of the sympathetic anlage are referred to as primitive sympathetic cells; in the 14–16 mm. stage, these cells differentiate into larger less basophilic cells which are referred to as phaeochromoblasts. The latter undergo further differentiation in foetuses of between 27 and 46 mm. with the formation of phaeochromocytes. This change is again characterized by a reduction in nuclear basophilia. Mitotic figures have been observed in primitive cells and phaeochromoblasts but not in phaeochromocytes.

Kohn (1903) observed ‘paraganglia’ in a 19·5 mm. embryo, and although he referred to changes in cell type and vascularity as development continued, the same term was retained throughout. Zuckerkandl (1912) made similar observations. Iwanow (1930, 1932) described the appearance of ‘paraganglia’ at the 30 mm. stage, but did not use this term to describe the groups of phaeochromoblasts which were observed in the same situations in embryos of between 17 and 30 mm. Kohn and

Zuckerkandl did not recognize the two-stage differentiation of phaeochromocytes, and referred to all collections of cells (other than neurones and their precursors) which are developmentally and topographically related to the sympathetic nervous system as 'paraganglia' or 'chromaffin bodies'. Iwanow included within this term only those structures which are morphologically similar to the 'paraganglia' of the older foetus and child, which are composed predominantly of phaeochromocytes, have a well-developed vascular network and which are probably functionally active. The second interpretation is accepted by the present writer.

The formation of para-aortic bodies is characterized not only by a differentiation of the cells but also by the appearance of a well-developed vascular network which is in the main composed of sinusoidal capillaries. Throughout intra-uterine life the structures have a rich blood supply derived from neighbouring arteries: in the early stages from the aorta, unpaired branches of the aorta, and lateral splanchnic arteries, and in the later stages from the aorta and unpaired branches, and derivatives of the lateral splanchnic vessels (testicular, ovarian and renal arteries). The venous drainage is into nearby veins.

The extension of primitive sympathetic elements into the adrenal glands is well recognized. Wrete (1927) observed the continuity between the chromaffin tissue of the cranial para-aortic bodies and the adrenal glands of foetuses of 21·1, 37·8 and 39·4 mm. During the present investigation three types of cells have been observed invading the adrenal glands:

(1) Primitive cells between the stages of 14 and 270 mm. (Pl. 1, fig. 2, Pl. 3, fig. 7).
(2) Phaeochromoblasts at the 19·5 mm. stage (Pl. 3, fig. 7).
(3) Phaeochromocytes at the 46–52 mm. stage (Pl. 4, fig. 9).

The phaeochromocytes of the bodies and adrenal medulla, after fixation in Bouin, formol-dichromate and Zenker, and staining with haematoxylin and eosin and a Masson trichrome method, are structurally identical.

These findings support the concept that the phaeochromocytes of the adrenal medulla and para-aortic bodies are identical developmentally and structurally.

In the course of the present work the differentiation of primitive sympathetic cells into sympathoblasts was first noted in the prevertebral ganglia of a 46 mm. foetus; differentiation of the cells of the gangliated chains was first observed at a later stage (92 mm.). The sympathoblasts are initially indistinguishable from phaeochromoblasts, but enlarge relatively quickly and develop at least one large nucleolus, after which the two types can be readily recognized. Phaeochromocytes were first observed in the ganglia of the sympathetic chains in a foetus of 108 mm. It would, therefore, appear that the primitive cells of the gangliated chains differentiate into both chromaffin and nervous elements at a later stage of development than those present in the prevertebral plexuses; this is in keeping with the observations of Kohn (1903) and Lucas Keene & Hewer (1927).

The close association between lymphoid tissue and chromaffin tissue in the human foetus does not appear to have been reported previously. Ivanoff (1925) reported a perivascular lymphoid infiltration of the chromaffin bodies of children aged 2–3 years. This was associated with hyaline and fibrous degeneration. The finding of para-aortic bodies in the centres of lymph nodes (Pl. 4, fig. 10) in foetuses of 108 and 150 mm. may be fortuitous, as sympathetic nerve fibres are commonly observed traversing lymph nodes at the same stage and in older foetuses. This explanation would also be adequate for the side to side apposition noted in older foetuses and in a 3 lb. 10 oz. infant, but does not explain the direct lymphocytic invasion and perivascular infiltration noted in the older specimens (Pl. 4, fig. 11). As associated signs of degeneration were not observed, the significance of these findings is not clear. The organs of Zuckerkandl were unaffected in all specimens.

It is generally accepted that macroscopic para-aortic bodies are not observed in the adult. Lucas Keene & Hewer (1927) observed an increase in fibrous tissue in the organs of Zuckerkandl of a full-term foetus and considered that the change was a sign of degeneration. Bonnamour & Pinatelle (1902) observed normal bodies in children of 5–6 years of age, but found that the appearances were variable. Zuckerkandl (1912) found microscopic evidence of degenerated chromaffin bodies in an adult aged 39 years and of hyaline degeneration of these structures in a child aged 2 years. Ivanoff (1925) reported that the para-aortic bodies reach maximum development in children between 12 and 18 months of age and that after that time atrophy occurred.

In the present investigation an increase in connective tissue stroma was observed in the larger bodies of foetuses of and above 150 mm. in length. This is not regarded as a sign of degeneration but as a normal response to the increase in bulk of the structures. Cellular degeneration was not observed during intra-uterine life.

Primitive cells are associated with the peripheral zones of all the para-aortic bodies of foetuses up to 150 mm. and disappear in the period between 150 and 270 mm. This disappearance coincides with the apparent cessation of growth of all bodies except the organs of Zuckerkandl. Mitotic figures have not been observed in microscopic sections of the para-aortic bodies of a 270 mm. foetus or a 3 lb. 10 oz. infant.

A slight increase in the size of the organs of Zuckerkandl towards the end of intra-uterine life has been observed during the present investigation, and agrees with the findings of Zuckerkandl (1901), Bonnamour & Pinatelle (1902) and Iwanow (1932). Bonnamour & Pinatelle (1902) stated that mitotic figures were not observed in these structures at this stage but described amitotic nuclear division. In the present series neither mitotic nor amitotic division has been observed, and the increase appears to be due in part to an increase in connective tissue stroma and blood vessels.

Zuckerkandl (1901), Bonnamour & Pinatelle (1902) and Iwanow (1930, 1932) described in detail the variations in shape of the organs of Zuckerkandl. Observations made during this investigation indicate that the cell whorls of the 16 and 19·5 mm embryos develop on a more or less bilaterally symmetrical pattern, without extension across the midline (Text-fig. 1, Pl. 2, figs. 5, 6). This pattern is followed initially by the large para-aortic bodies of the smaller foetuses (Text-figs. 2, 3), but as the primitive cells at the periphery of these structures differentiate, the organs of Zuckerkandl commonly unite across the midline with the formation of an isthmus. An isthmus was first observed in a 142 mm. foetus (Text-fig. 6) and is present in five out of seven of the older specimens examined.

Phaeochromocytes have been observed singly and in small non-encapsulated groups in the sympathetic ganglia (prevertebral and of the sympathetic chains) in foetuses of 108 and 270 mm. and in a 3 lb. 10 oz. premature infant. Only one small para-aortic body was observed in relation to the gangliated chains of all specimens examined; this was found in a foetus of 270 mm. These findings indicate that the para-aortic bodies in the abdomen of the human foetus are largely confined to the prevertebral plexuses. Zuckerkandl (1912) wrote: 'These bodies may occur wherever there are sympathetic ganglia.' This statement may well be extended in the light of the present work to read that the para-aortic bodies are found in all parts of the prevertebral sympathetic plexuses of the abdomen and pelvis and less commonly in association with the sympathetic chains.

SUMMARY

1. The adrenal medulla and extra-adrenal chromaffin tissues of the human foetus develop from sympathetic elements.

2. Primitive sympathetic cells situated in the pre-aortic region begin to differentiate into phaeochromoblasts between the 14 and 16 mm. stages. The phaeochromoblasts are arranged in whorls. In the peripheral zones of the whorls primitive cells may be observed.

3. Between the 27 and 46 mm. stage the phaeochromoblasts undergo further differentiation with the formation of phaeochromocytes. This change is accompanied by an increase in the vascularity of the structures, and the whorl-like formation of cells is replaced by cell cords. From this stage onwards the structures are referred to as para-aortic bodies.

4. Primitive sympathetic cells, phaeochromoblasts and phaeochromocytes have been observed extending into the adrenal glands.

5. All para-aortic bodies except the organs of Zuckerkandl appear to attain a maximum size between the 150 and 270 mm. stages. The organs of Zuckerkandl continue to enlarge up to the end of intra-uterine life. Para-aortic bodies may be found in all parts of the abdominal and pelvic prevertebral sympathetic plexuses, and less commonly in association with the sympathetic chains.

6. A close association between lymphoid tissue and para-aortic bodies has been noted in foetuses of and after the 108 mm. stage.

In conclusion I should like to thank Prof. A. Durward for his advice, encouragement and instructive criticism throughout this investigation; the staffs of the Departments of Gynaecology and Pathology, St James's Hospital, Leeds, who have provided the foetal material; Prof. R. A. Willis, who allowed me to examine an 8 and a 12 mm. embryo; and Prof. W. A. Bain and the staff of the Department of Pharmacology for advice and facilities placed at my disposal during the performance of bio-assays.

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EXPLANATION OF PLATES

Abbreviations

AO, aorta; *AD*, adrenal gland; *LA*, lateral splanchnic artery; *PAB*, para-aortic body; *PB*, phaeochromoblasts; *SN*, primitive sympathetic cells and fibres; *SP*, primitive sympathetic cells.

PLATE 1

Fig. 1. Transverse section of an 8 mm. human foetus taken immediately cranial to the origin of the coeliac artery. The sympathetic anlage forms a chain on the dorso-lateral aspect of the abdominal aorta. The rudiment of the developing adrenal gland is closely related to columnar cells (*C*), which line the adjacent coelom. Haematoxylin and eosin. × 140.

Fig. 2. Section of a 14 mm. human foetus at the level of the origin of the coeliac artery. Primitive sympathetic cells and nerve fibres are invading the medial aspects of the adrenal glands. Haematoxylin and eosin. × 130.

Fig. 3. Transverse section of a 14 mm. human foetus mid-way between the origins of the superior and inferior mesenteric arteries. The pre-aortic plexus is composed entirely of primitive cells and nerve fibres. Haematoxylin and eosin. × 140.

Fig. 4. Transverse section of a 16 mm. human foetus mid-way between the origins of the superior and inferior mesenteric arteries, showing a pre-aortic whorl of phaeochromoblasts. Haematoxylin and eosin. × 140.

PLATE 2

Fig. 5. Transverse section of a 19·5 mm. human foetus between the origins of the superior and inferior mesenteric arteries—nearer the former. Whorls of phaeochromoblasts lie in the pre-aortic region, primitive cells are found at the periphery of these structures. Haematoxylin and eosin. × 140.

Fig. 6. Transverse section of a 19·5 mm. human foetus immediately cranial to the origin of the inferior mesenteric artery, showing two large whorls of phaeochromoblasts. Haematoxylin and eosin. × 140.

PLATE 3

Fig. 7. Transverse section of a 19·5 mm. human foetus between the origins of the coeliac and superior mesenteric arteries. Sympathetic nerve fibres and primitive cells are invading the medial aspect of the adrenal gland. A few phaeochromoblasts may also be observed extending from the lateral whorl of phaeochromoblasts into this structure (*EXT*). Haematoxylin and eosin. × 140.

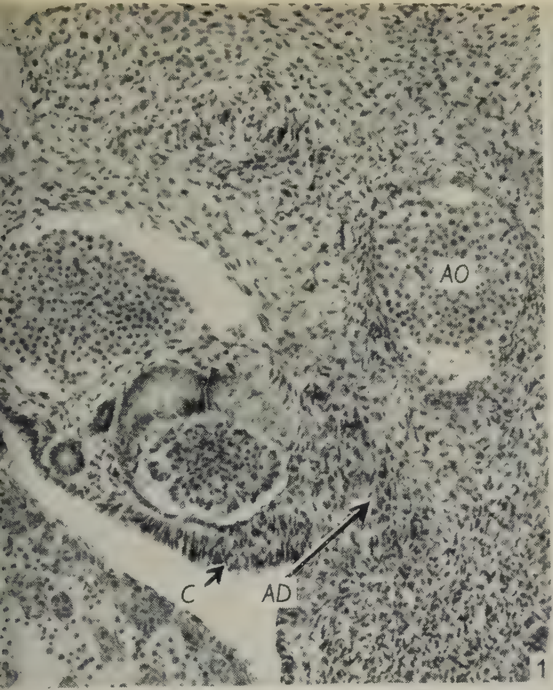
Fig. 8. Transverse section of a 46 mm. human foetus between the origins of the superior and inferior mesenteric arteries. Two large para-aortic bodies are situated in the pre-aortic region on each side of the mid-line. Primitive sympathetic cells may be observed in the peripheral zones of one body. A lateral splanchnic artery is supplying the body on the left. Haematoxylin and eosin. × 130.

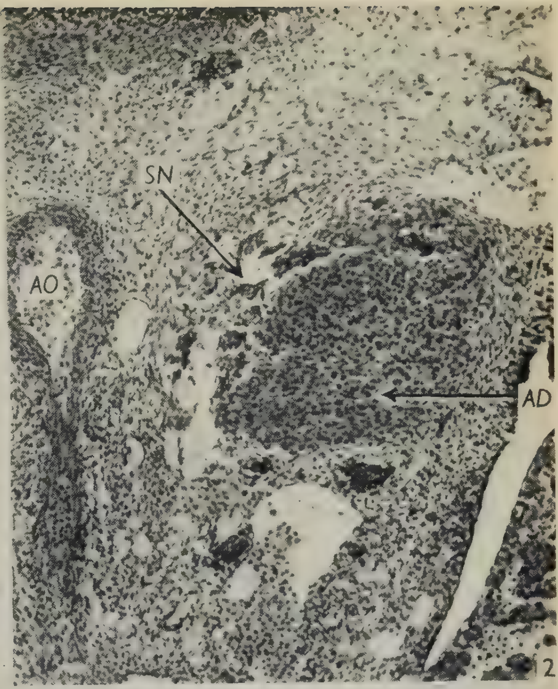
PLATE 4

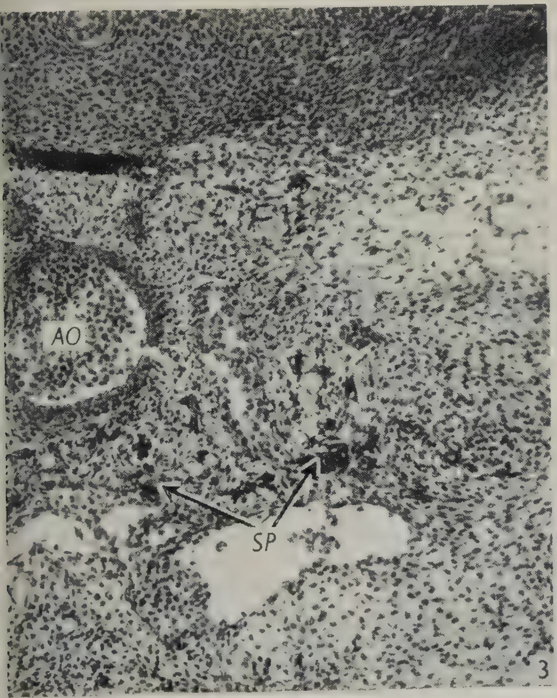
Fig. 9. 52 mm. human foetus. Shows the medial aspect of the right adrenal gland with associated para-aortic bodies and primitive cells. Phaeochromocytes may be traced from the largest para-aortic body into the adrenal gland. Masson. × 140.

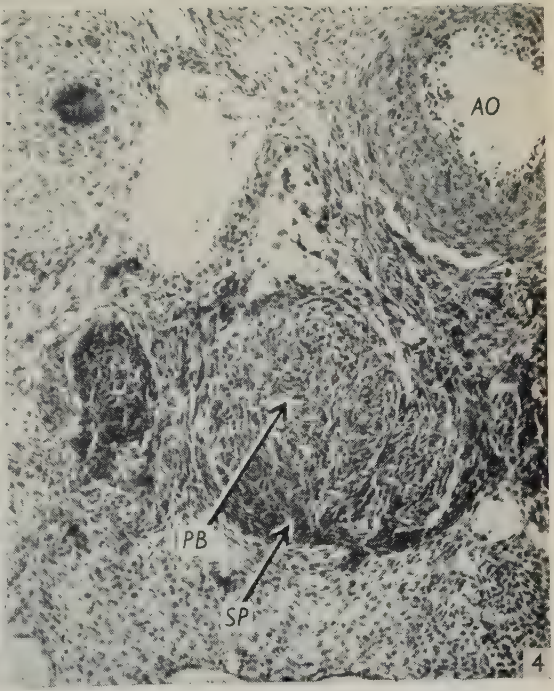
Fig. 10. 108 mm. human foetus. A para-aortic body may be observed lying inside a developing lymph node. Haematoxylin and eosin. × 160.

Fig. 11. 270 mm. human foetus. A large para-aortic body (right) is in contact with a mass of lymphoid tissue. Lymphocytes are infiltrating the para-aortic body. Haematoxylin and eosin. × 160.

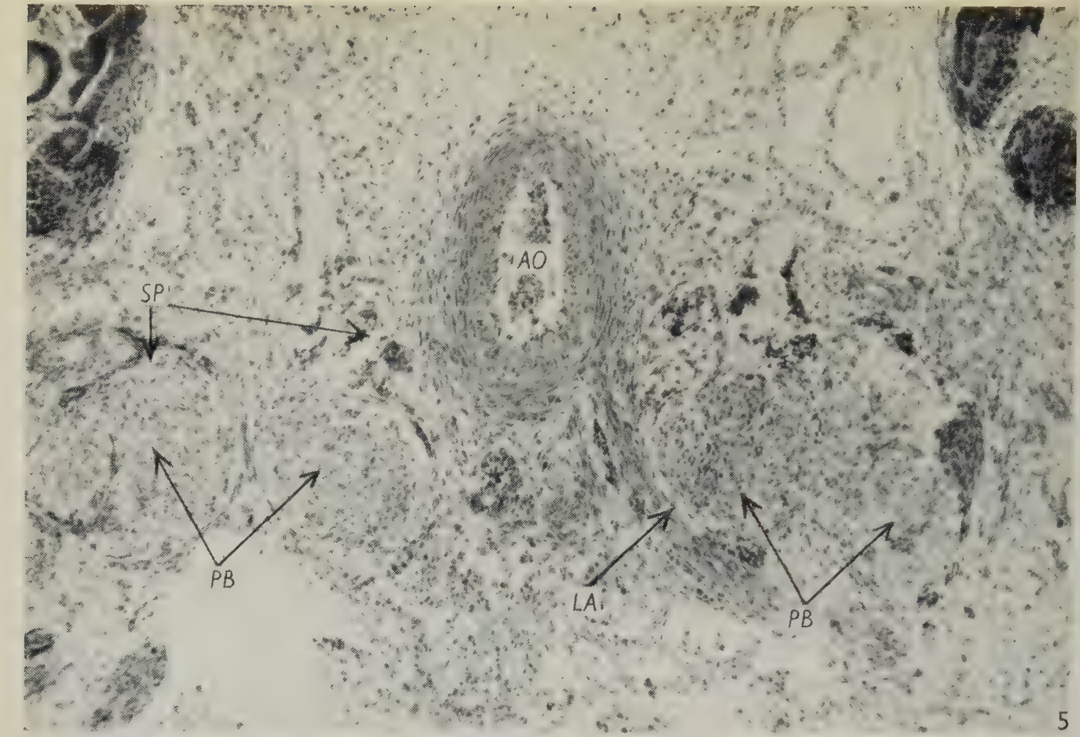


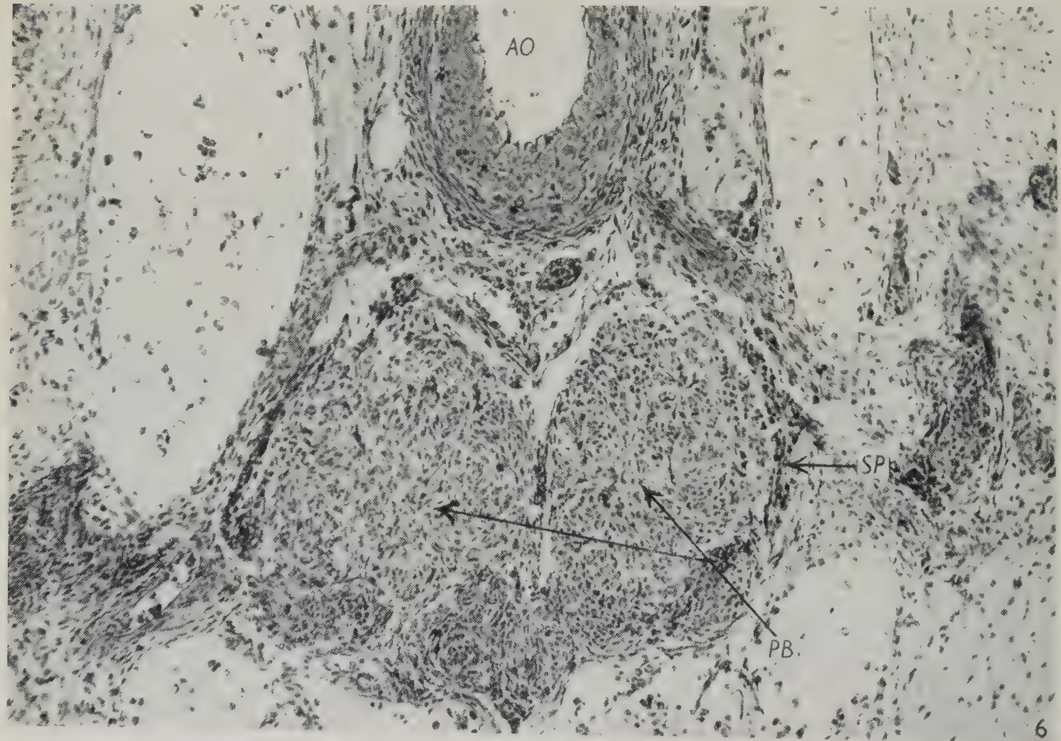




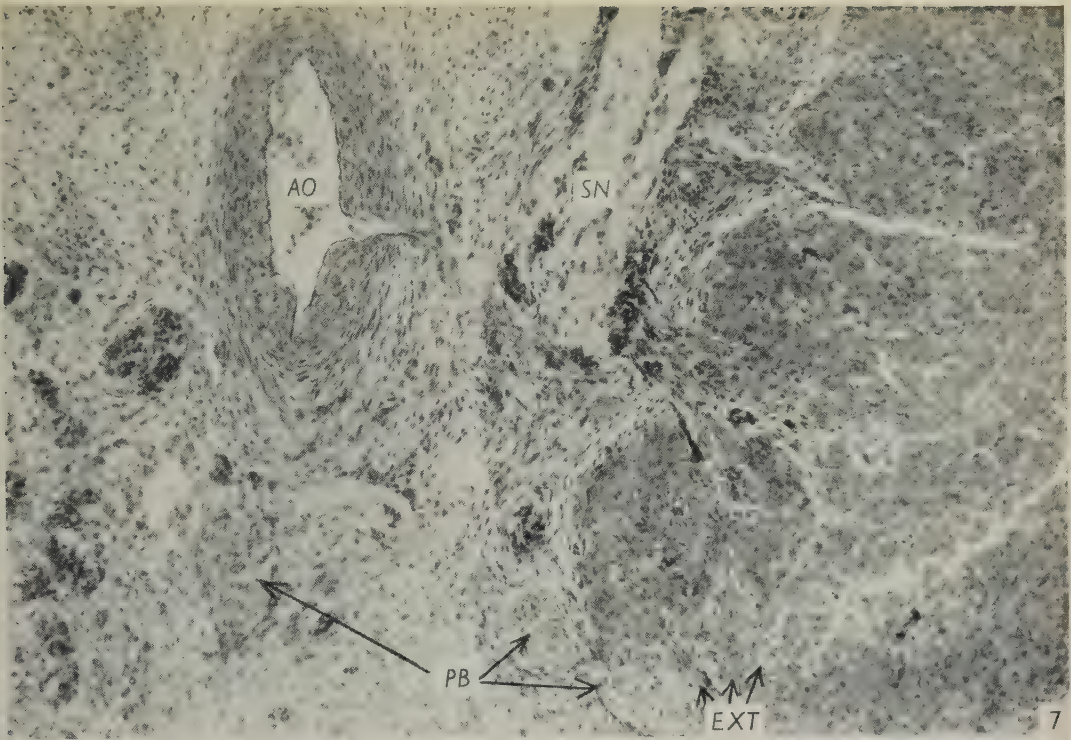


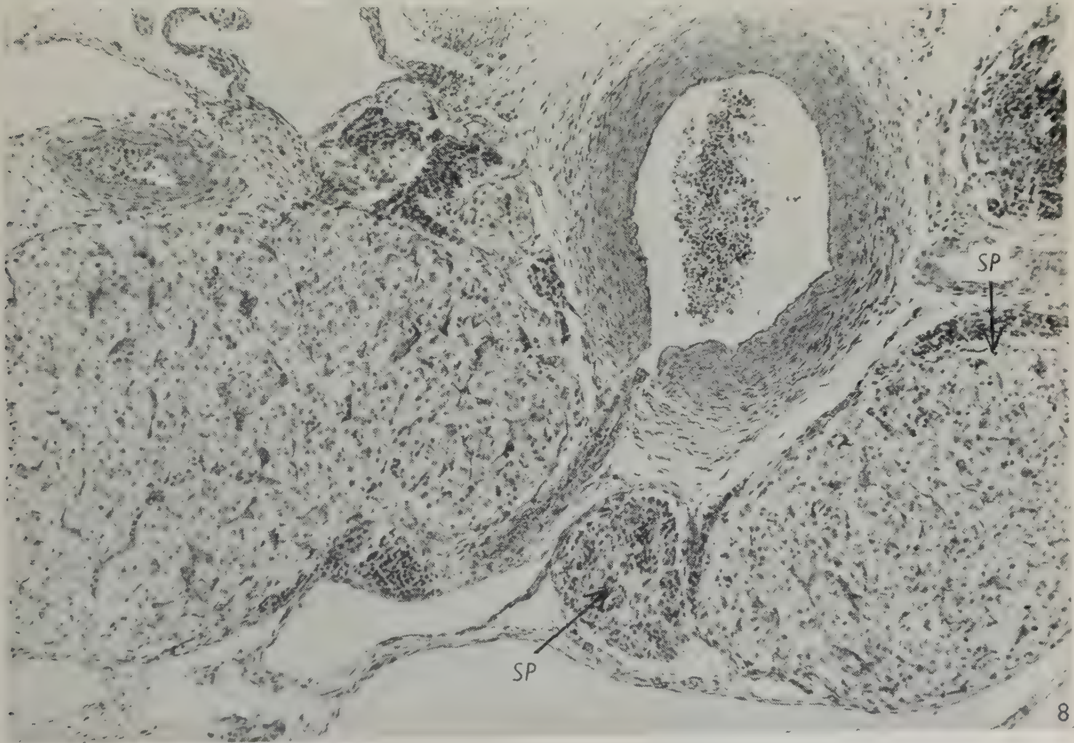
COUPLAND—The prenatal development of the abdominal para-aortic bodies in man



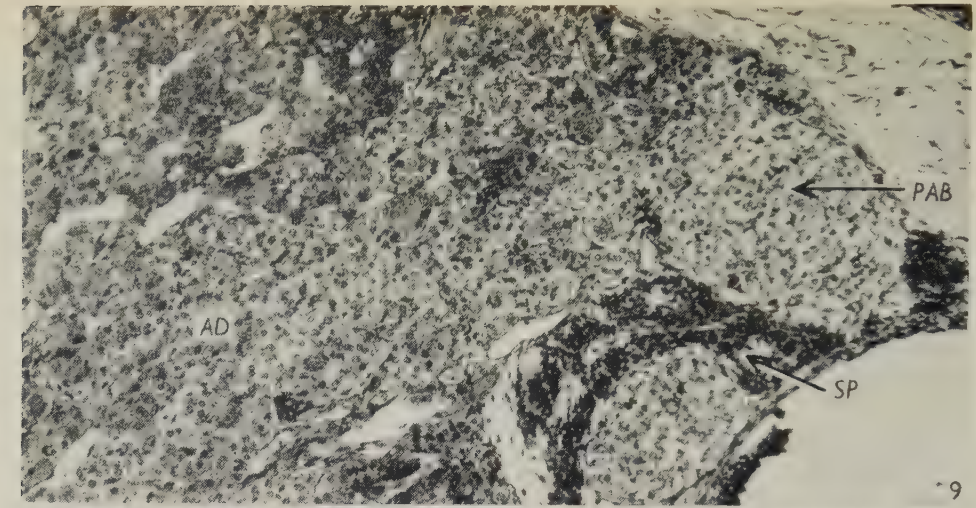


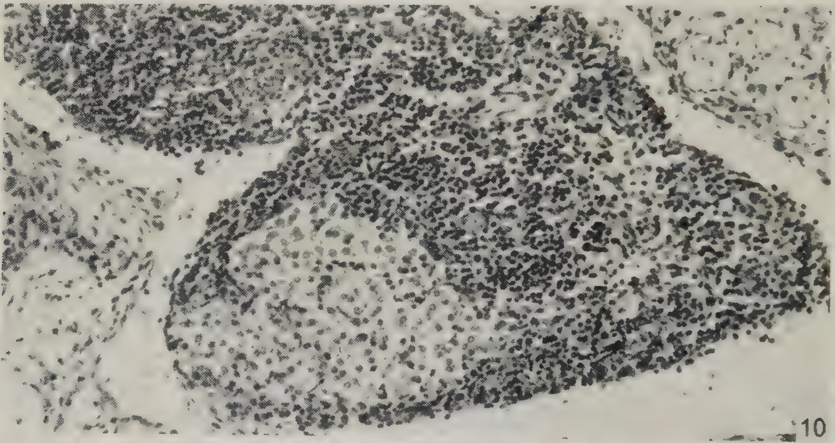
COUPLAND—THE PRENATAL DEVELOPMENT OF THE ABDOMINAL PARA-AORTIC BODIES IN MAN

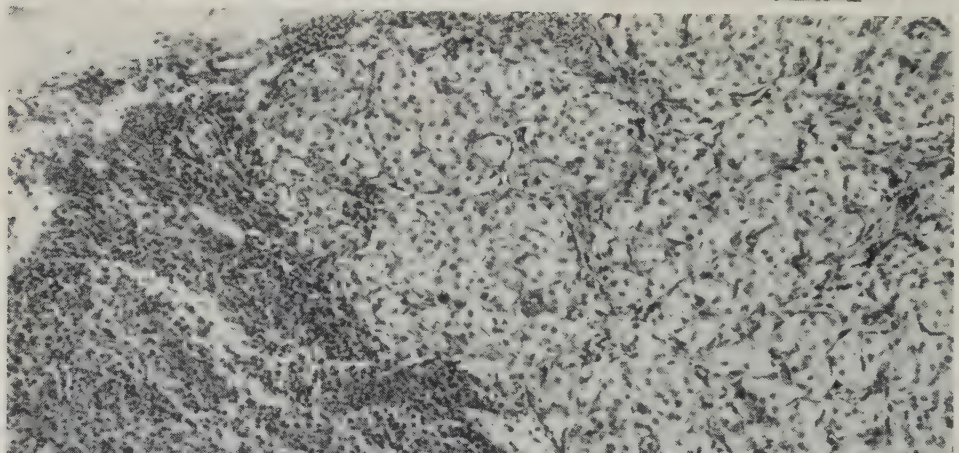




COUPLAND—The prenatal development of the abdominal para-aortic bodies in man







COUPLAND –THE PRENATAL DEVELOPMENT OF THE ABDOMINAL PARA-AORTIC BODIES IN MAN

STUDIES ON THE MORPHOGENESIS OF THE MOUSE STERNUM

I. NORMAL EMBRYONIC DEVELOPMENT

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INTRODUCTION

Although the developmental mechanics of avian limb bones has been widely studied (Strangeways & Fell, 1926; Fell, 1928; Fell & Robison, 1929; Fell & Canti, 1934; Warren, 1934; Fell & Landauer, 1935; Murray, 1936; Hamburger, 1938; Rudnick, 1946; Rawles, 1947; Saunders, 1948), little experimental work has been done on the morphogenesis of other skeletal rudiments in warm-blooded animals. It seemed to the author that the mammalian sternum might prove particularly interesting material for investigations of this type, in view of the complexity of its structure and its remarkable adaptations to functional requirements in different species. An extensive series of observations and tissue culture experiments were accordingly made on the foetal mouse sternum, and the results are described in the following series of papers.

Previous work on the development of the sternum was mainly concerned with the controversy over its origin (e.g. Hanson, 1919; Gladstone & Wakeley, 1932) and was entirely descriptive. The first experimental studies on the origin and development of the sternum were made by Fell (1939) in birds; using the tissue culture technique, she showed that the avian sternum originates independently of both the ribs and the pectoral girdle, that the sternal rudiments are capable of considerable self-differentiation *in vitro* and that the primary factor responsible for the median movement and fusion of the two bilaterally situated rudiments is the migration of undifferentiated tissue in the dorso-lateral body-wall.

In the mammal, the sternum appears as two mesodermal bands in the dorsolateral body-wall; these bands lengthen in a caudal direction, move towards each other and eventually fuse in the mid-ventral line. The double rod thus formed is attached to seven pairs of ribs; hypertrophy of the cartilage cells, followed by ossification, takes place in the intercostal regions so that the sternum acquires a segmental structure. The present investigations were undertaken with three main objects: (1) to determine the origin of the sternum, i.e. whether it is formed from the ribs, the pectoral girdle or develops independently in the mesoderm; (2) to ascertain the relative importance of intrinsic and extrinsic factors in its development; (3) to find by what mechanism the two dorsolateral mesodermal bands move to the mid-ventral position to form the double rudiment of the sternum.

This paper (Part I) is the first of a series of papers dealing with the normal embryology of the mouse sternum. Although the anatomical development of the mammalian sternum has often been described (Hanson, 1919; Gladstone & Wakeley, 1932; Bryson, 1945), so far no systematic account has been given of the associated histological changes and of how these are correlated with the gross morphogenesis of the bone. A detailed study of the anatomical and histological development of the normal mouse sternum was therefore made to provide a basis for the experimental work to be described in Parts II & III.

MATERIAL AND METHODS

Twenty-five mouse embryos, from 12 days up to the time of birth, and two infant mice, 1-day old, were used. For serial sections, the material was fixed in 3–4 % acetic Zenker's solution, formol-alcoholic solution or Carnoy's mixture, embedded in paraffin and cut at 8 μ. Most of the sections were stained with Ehrlich's haematoxylin and chromotrope 2R; for others, Delafield's haematoxylin followed by chromotrope 2R or Biebrich scarlet was used. For the demonstration of intercellular fibres, Heidenhain's azan staining technique was employed.

For whole mounts, the sternal regions were dissected out, fixed in 4 % acetic Zenker's solution or 5 % acid alcohol and stained with carmalum or dilute haematoxylin. After dehydration they were cleared in xylol, oil of winter green or methyl salicylate and mounted in Canada balsam or D.P.X.

OBSERVATIONS

(*a*) *The origin of the sternum*

Before describing the first appearance of the primary sternal bands, it is necessary to consider certain changes in the thoracic body-wall which immediately precede the formation of the bands (Pl. 1, figs. 1–4).

In a 12^--day embryo, the ventral thoracic wall and the lateral wall up to the level of the ventral surface of the fore-leg bud are composed of a loose stellate reticulum which is undifferentiated (Pl. 1, figs. 1, 2). The dorso-lateral wall, from the ventral surface of the leg-bud to the roof of the thoracic cavity, consists mainly of differentiated tissue which in the leg-bud region comprises the procartilaginous pectoral girdle, including two short, blunt clavicular rudiments, and the musculature (Pl. 1, fig. 1). Behind the limb, the dorso-lateral wall is mainly occupied by the segmental muscles and the costal processes. The latter, which do not extend as far ventrally as the muscles, consist of compact procartilage; their distal ends are sharply defined from the surrounding tissue and they show no sign of terminal expansion or proliferation (Pl. 1, fig. 2*a*).

Apparently emigrating from the differentiated dorso-lateral tissue is a stream of undifferentiated, spindle-shaped cells which are invading the stellate reticulum of the lateral walls. This stream has advanced only a short distance below the base of the leg-bud (Pl. 1, fig. 2*b*).

A few hours later (12 days) all the components of the dorso-lateral tissue have moved further ventrally (Pl. 1, figs. 3, 4). The clavicles have elongated and become more distinct, and the musculature and the ribs now extend below the base of the leg-bud in the fore-part of the thorax. At the level of the clavicles, the 'advance guard' of spindle-shaped cells from either side have met in the mid-ventral line to form a broad, continuous band across the body-wall (Pl. 1, fig. 3). A short distance farther back, however, the two streams are still widely separated by the original stellate reticulum.

The costal processes are even more definite in outline than before, and are sharply demarcated from the stream of downward migrating, spindle-shaped cells immediately below their distal ends (Pl. 1, fig. 4).

Correlated with this downward movement of the dorso-lateral tissue, remarkable changes occur in the original ventro-lateral body-wall and also in the shape and size of the thoracic cavity. These changes will be described later in a separate section.

The paired sternal rudiments are first recognizable in a 12½-day embryo (Pl. 1, fig. 5). Each arises as a rod-like condensation in the dorsal part of the stream of spindle-shaped cells, immediately below the ventral ends of the ribs. They are first distinguishable from their articulation with the clavicles, backwards to about the level of the first sternal rib. Each band is triangular in cross-section, the apex of the triangle pointing in a median direction. The median surface of each bar is directly continuous with the stream of spindle-shaped cells so that it is impossible to determine the boundary of the chondrogenic tissue. The cells composing the bars are still somewhat elongated and triangular in outline and are orientated in the same direction as the spindle-shaped cells.

Behind the level of the first sternal rib, the rod becomes smaller and more indistinct and at the level of the third sternal rib is so diffuse as to be almost unrecognizable.

The ribs, which have now begun to chondrify, remain sharply delimited from the surrounding tissue, and there is no indication that they are concerned in the formation of the sternal bands which appear to develop quite independently in the underlying mesoderm.

The clavicles at this stage have well-ossified shafts but their distal ends still consist of late procartilage.

(*b*) *Changes in the thoracic wall*

As previously stated, the downgrowth of the dorso-lateral tissue, including the musculature, skeletal rudiments and connective tissue, is associated with striking histological changes in the thoracic wall. Three processes are concerned: (*a*) the invasion of the reticular mesenchyme of the ventral body-wall, first by an 'advance guard' of spindle-shaped cells from the dorso-lateral region and then by the muscle and skeletal structures; (*b*) the appearance of pronounced cell degeneration in the ventral wall involving first the original mesenchyme, and subsequently the down-growing tissue; (*c*) the gradual contraction of the original, degenerating ventral body-wall as it is replaced by the downgrowing dorso-lateral material.

These processes proceed in a wave which spreads backwards from the clavicular region. Thus dorso-lateral downgrowth and ventral degeneration first appear in the extreme anterior end of the thorax, and a short distance posteriorly the thoracic wall is unaltered. As the two streams of dorso-lateral tissue approach each other, the ventral body-wall becomes folded; when the tissues from either side meet, the second wave of degeneration, in the form of a sharply defined median tract, appears. Meanwhile, mesenchymal degeneration becomes most noticeable farther back. With the complete fusion of the sternal bands in front, the folding of body-wall and the median degeneration vanish from this region but reappear more caudally where fusion of the sternal bands is about to take place. This continues until the downgrowth of the dorso-lateral tissue is complete throughout the length of the thorax.

In the extreme anterior end of the thorax the combined effects of this downgrowth, degeneration and contraction cause the gradual obliteration of the anterior part of the pericardial cavity, so that the anterior pericardial wall, which at first lies in front of the clavicular rudiments, gradually retreats to the level of the second sternal ribs (cf. Pl. 1, figs. 1, 3 and 8).

The details of these histological processes will now be described.

Ventral degeneration has already appeared at the 12^{-}-day stage, but is not yet very advanced. Only a few necrotic cells are found in the skin and mesenchyme in the fore-part of the thorax. At this stage the pericardial cavity extends well in front of the short, procartilaginous clavicles, the distal ends of which are widely separated (Pl. 1, fig. 1).

As the dorso-lateral tissue moves downwards, the degeneration increases. In a 12-day embryo, necrosis of mesenchymal cells of the thoracic floor has spread to the level of the first sternal rib, while in front the first sign of the second wave of degeneration of cells is seen. These cells, which are in the initial stages of degeneration, are scattered over a fairly narrow, mid-ventral tract at the level of the clavicles (Pl. 1, fig. 3).

In a 12½-day embryo, the stage at which the sternal bands are first clearly recognizable, the dorso-lateral tissue from either side nearly meets in the most anterior part of the thorax (Pl. 1, figs. 6, 7). Thus the distal ends of the clavicles are almost in contact and are only separated by a narrow mass of cells, many of which have degenerated. Immediately below the clavicles the two streams of spindle-shaped cells now form a continuous band uniting the musculature of opposite sides. The pericardial cavity has been reduced in the clavicular region as a result of the downgrowth and is relatively small in cross-section (Pl. 1, fig. 6). A few sections backwards the sheet of spindle-shaped cells uniting the sternal and muscular rudiments of opposite sides forms a kind of trough enclosing the necrotic pericardial membrane. Below the sheet of flattened cells, cells are crowded and necrotic along the median line (Pl. 1, fig. 7).

Median degeneration in the ventral skin and mesenchyme has now spread farther back, and is almost as advanced at the level of the first sternal ribs as it was at the extreme anterior end of the thorax at the 12-day stage. At the level of the third sternal rib, the mesenchymal body-wall is slightly necrotic, as the first wave of necrosis has only just reached this region.

By about the thirteenth day the anterior end of the pericardium has been pushed a little farther backwards until it is almost opposite the first sternal ribs. The two streams of spindle-shaped cells have united to form a continuous sheet which is fairly distinct as far as the third sternal rib. Near the level of the first sternal rib, the mid-ventral skin and connective tissue are beginning to recover from the degeneration seen in the 12^{+}-day embryo. The second wave of necrotic changes along the mid-line is fairly marked as far back as the third sternal rib, while the mesenchymal degeneration is seen as far as the hind-end of the sternal rudiment.

A few hours later the anterior pericardial wall has retreated to a position about mid-way between the first and second sternal ribs (Pl. 1, fig. 8). As described in later sections, the anterior ends of the sternal bands are in contact at this stage, but the bands and their associated musculature begin to diverge widely behind the level of the fourth sternal rib.

The median body-wall between the muscles shows a curious structure in the anterior part of the thorax (Pl. 1, fig. 8). In transverse section the cells, blood vessels and intercellular fibres radiate outwards from the point of union of the muscles, as if they had been bunched together dorsally by the rapid ingrowth of the sternal and muscular rudiments. Although there is relatively little cell degeneration in this area, small haemorrhages occur in places, showing that the disturbance of the tissue has been sufficiently rapid and profound to rupture some of the smaller vessels. Farther back, where the sternal bands and muscles diverge more widely, this radiating structure becomes less and less conspicuous, and towards the hind-end of the sternum the median tissue shows the usual orientation parallel with the skin.

At the level of the second and third sternal ribs, the ventral sheet of spindle-shaped cells uniting the muscular and sternal rudiments of opposite sides is reduced to a necrotic mass compressed between the approaching rudiments. The maximum abnormality in the mid-ventral skin is at the level of the fourth sternal rib, where the sternal bands are on the point of uniting. Here the median degeneration is most advanced with numerous necrotic cells between the approaching elements from either side. Both the pericardium and the epidermis are thrown into deep papilliform, and very necrotic folds (Pl. 2, fig. 9). Behind the fourth sternal ribs, as the sternal bands and muscles diverge more widely, the sheet of flattened cells connecting them is still distinct. Above the sheet, the pericardial membrane is folded, but becomes more normal towards the hind-end of the sternum where the bands are still far apart.

The histological changes in the thorax have nearly ended by the fifteenth day. The pericardium is normal throughout its length, and although the muscles from opposite sides are still not quite in contact, degenerated cells have largely disappeared from the intervening tissue which is now fibrotic. Traces remain of the radiating structure described above in the median body-wall, but this structure is less conspicuous than before. Up to the level of the fifth sternal rib the median ectoderm is almost normal. Behind this level, however, it shows the same deep foldings which were observed in the 13½-day embryo at the level of the fourth sternal ribs. The folds diminish in size near the sixth sternal ribs, but are recognizable as far as the posterior end of the sternum.

In the 17-day embryo the histological processes described in this section appear to be complete.

(*c*) *The development of the sternum*

The development of the sternum was followed from its first appearance in a 12½-day embryo to the stage when it assumes all the characteristics of the adult structure in a newborn mouse. Although the post-coitus age of the embryos could be roughly determined by observing the formation of the copulation plug and checked by Grüneberg's (1943) description of the external features, there were always variations in the degree of development of embryos of the same litter. It was thus found more practical to describe the development as a series of numbered stages, the approximate age at which each stage is reached being given.

Stage 1. As described in §(*a*), the sternal rudiment first appears at about the twelfth day (Pl. 1, fig. 5), as a pair of mesodermal condensations in the dorso-lateral body-wall amongst a stream of spindle-shaped cells. These mesodermal bars are immediately below, but independent of, the distal ends of the ribs and are just distinguishable from their articulation with the clavicles, backwards to about the level of the first sternal rib; they are triangular in cross-section, are composed of elongated cells of irregular outline and their median surface is continuous with the stream of spindle-shaped cells.

Stage 2. At about the thirteenth day the sternal bars become more distinct and are clearly distinguishable down to the level of the fifth sternal rib. They have also moved toward each other in a median direction. Behind this level the sternal rudiments are still far apart (Pl. 2, fig. 10).

In transverse sections, the sternal bars remain roughly triangular in shape and are still continuous with the stream of flattened cells. The latter now form a thick continuous sheet uniting the muscular and sternal rudiments of opposite sides. The main body of the sternal bars is composed of procartilage (Pl. 2, fig. 11).

The ribs, which have chondrified, have extended further ventrally.

Stage 3. A few hours later the sternal bars can be traced back to the level of the seventh sternal rib and are much closer together. They have also begun to assume their characteristic anatomical structure. At the cranial end the omosternum can be seen bending laterally to form a transverse process occupying the space between the clavicle and the sternal end of the first rib. In the region of the presternum, just in front of the first rib, the two bars have nearly fused but they are still separate at the level of the mesosternum; the xiphisternum is hardly recognizable.

Transverse sections show that at the level of the clavicle the sternal rudiments, which are almost in contact, are bridged medially by the remains of the zone of flattened cells, forming a ridge to which the pectoral muscles are attached. A few sections backwards the distal ends of the first pair of sternal ribs are seen, below and in contact with the ventral surface of the sternal bars (Pl. 1. fig. 8); it is interesting to note that at this stage the ribs and the sternum are in more intimate contact than before and the boundaries between them can only be detected by very careful observation. The second and third pairs of ribs, however, are clearly demarcated from the sternum by a layer of flattened chondroblasts.

The sternal rudiments become ill-defined and slope away from each other behind the level of the fourth sternal rib, while the sheet of flattened cells remains distinct.

Stage 4. In an embryo of about 14 days, the sternal bands have fused from the anterior end down to the level of the third rib, below which they still diverge. The gap becomes wider caudalwards and the forked posterior ends extend below the level of the seventh rib as the rudimentary xiphisternum (Pl. 2, fig. 12).

Frontal sections show clearly an intimate connexion between the ribs and the sternum (Pl. 2, fig. 12). The first sternal ribs are once more clearly demarcated from the sternal tissue by intervening layers of flattened cells; the ends are enlarged and slightly bifurcated. The second and third pairs of ribs seem to have pushed into the sternal tissue. There are layers of concentrically arranged small cells around each rib tip, some of which are in mitosis, and it is difficult to determine whether these layers of cells belong to the sternum or to the rib. The sternal tissue between the attachments of two consecutive pairs of ribs, i.e. the intercostal region, is much broader as compared with the sterno-costal junctions. From the fourth rib caudalwards, where the sternal bands have not yet fused, the pressing effect of the ribs on the sternal tissue is not so prominent, and it is much easier to distinguish between the two structures. Even here, however, the sternal bands have a slightly wavy appearance owing to the fact that they are rather closer together where the ribs are attached than in the intercostal regions.

The fact that the sternum becomes constricted at intervals corresponding to the sterno-costal junctions indicates that the growth of the ribs has exerted some pressure on the sternal tissue. It is also obvious that this force is greatest where the sternal bands have just fused, less where they are about to fuse, and least where they have been fused for some time, i.e. at the attachment of the first pair of ribs where the sternal tissue seems to have recovered from the effects of the pressure and has increased in width (Pl. 2, fig. 12).

Where fusion of the bars is complete, the sternum is now cartilaginous; the round or polyhedral chondroblasts show no definite arrangement but are evenly spaced with little matrix between them (Pl. 2, figs. 13, 14). From the level of the fifth sternal rib onwards, the diverging sternal bands are still composed entirely of procartilage (Pl. 2, fig. 15).

A definite perichondrium covers the whole sternum; it is more easily distinguishable in the first three or four segments.

Stage 5. By this stage (16-day) the fusion of the sternal bands is complete, forming a continuous bar of cartilage with all the anatomical structures present (Pl. 3, fig. 16). Its anterior end (the presternum) forms a broad head with lateral processes (omosterna) occupying the space between the inner ends of the clavicle and first rib. The main body of the sternum (the mesosternum) is a slender bar to which are attached the next six pairs of ribs (second to seventh inclusive). Caudal to the attachment of the last pair of ribs, a posterior process is formed with a slightly broader end (the xiphisternum).

In coronal sections signs of segmentation are seen (Pl. 3, fig. 16*a*); the cartilage cells have begun to hypertrophy in the intercostal regions but not at the sterno-costal junctions. In the hypertrophic areas the cells have lightly stained nuclei and vacuolated cytoplasm (Pl. 3, fig. 16*b*), and there is also an increase in the basophilic intercellular matrix in which fibrils can be easily demonstrated by azan staining.

This segmental hypertrophy starts from the anterior end of the sternum and spreads caudally into every intercostal region. There are six areas of hypertrophy (Pl. 3, fig. 16*a*): the first just below the presternum which remains cartilaginous, the next four in the next consecutive intercostal segments and the last in the xiphoid process. The most posterior portion of the latter remains small-celled. No hypertrophy occurs in the sixth intercostal segment.

In each intercostal region hypertrophy begins in the centre of the segment and spreads radially; it is most advanced in the first intercostal segment where the cells are larger and more matrix is present. In the following segments, hypertrophy is less intense and more localized. It is noteworthy, however, that the xiphoid process does not follow the order of position in being least advanced in hypertrophy, but is at a stage almost identical with that of the first intercostal segment. In the xiphoid process the dual origin of the centre of hypertrophy can be clearly seen; this is not apparent in the other anterior segments. This process also appears to lie in a plane a little dorsal to that of the anterior portion of the sternum so that it is impossible to show the whole sternum in a frontal section at this stage (e.g. Pl. 3, fig. 16*a*).

At the sterno-costal junctions, growth or some rearrangement of cells must have occurred (Pl. 3, fig. 16*b*). Instead of forming a narrow strip compressed between the penetrating rib tips of both sides, as at an earlier stage, the sternal tissue in this region has regained its normal width and appears to be even broader than the intercostal region. The demarcation between sternal and rib tissue again becomes obvious, and a clearly defined sheath of flattened chondroblasts separates the sternum from the rib tips. The histological appearance of the sterno-costal junction is very different from that of the intercostal regions. At the junction the cells remain very small and are arranged in three groups: a central area of scattered chondroblasts, with many intercellular fibrils but no basophilic matrix, is sandwiched between two lateral groups of very small, closely packed cells situated next to each rib tip.

The segmental hypertrophy of the cartilage cells is preparatory to ossification, the first sign of which can be detected at this stage (Pl. 3, fig. 17). It appears in the first two or three segments and in the xiphisternum, where the perichondrium has become transformed into a two-layered periosteum composed of an outer layer of greatly flattened fibroblasts and an inner layer of more oval-shaped osteoblasts; the latter is sharply delimited from the deeply stained cartilage by a thin layer of bone.

Stage 6. The next stage (18-day) in the development of the sternum is chiefly histological and the main feature is ossification; there is also a general enlargement and some anatomical differentiation of the xiphisternum (Pl. 3, figs. 18, 19). Periosteal ossification forms a collar round the cartilage in the usual way and endochondral ossification proceeds in the body of each segment. There are six ossification centres, each corresponding to one of the areas of chondroblastic hypertrophy described in stage 5. The hypertrophic cartilage soon becomes calcified and invaded by vascular mesenchyme which penetrates the periosteal bone; the erosion of the calcified cartilage is followed by the formation of cancellous bone.

In the first five segments, the degree of ossification follows the order of position, being most advanced in the first segment and progressively less so in the succeeding ones. The ossification centre in the xiphisternum, however, like the state of hypertrophy described in the previous stage, is as far advanced as in the first segment.

Seen in frontal sections, the first segment (Pl. 3, fig. 18) can be divided into several zones according to the state of the cells. In the middle of the segment there is an area which is eroded; above and below this are the large vacuolated hypertrophic cartilage cells, next to which, toward each end of the segment, is a zone in which the intercellular matrix stains more intensely basophilic and the cells are becoming increasingly compressed. Cells of the outermost layers are very flat and arranged elliptically. There is a well-developed collar of bone which, except where it is interrupted by excavation, is thickest in the centre and tapers away towards the two ends. Outside the collar the two-layered structure of the periosteum is very clear. The fibroblastic layer is fairly uniform in thickness, while the osteoblastic layer is thicker in the central part of each segment and thinner towards the two ends (Pl. 3, fig. 18).

The same arrangement of the cells in different stages of ossification can be found in the next four segments, but the area of erosion becomes smaller and more closely restricted to the centre. Periosteal bone-formation, too, is at an earlier stage; the bone collar is very thin and short in the fourth segment and hardly visible in the fifth. Transverse sections show that erosion begins in the middle of the segment by the invasion of vascular mesenchyme through the middle of either the dorsal and ventral or the lateral surfaces. The invading elements from the two sides meet in the centre and soon spread both dorso-ventrally and antero-posteriorly.

The sterno-costal junctions have undergone little change since the previous stage.

The xiphisternum has elongated appreciably and acquired a more plate-like shape than the mesosternum (Pl. 3, fig. 19). Chondroblastic hypertrophy and ossification are at a stage comparable with that in the first segment. A thick bone collar with well-developed periosteum invests the anterior half of the xiphisternum, and erosion has begun on the ventral border of the curved plate. The arrangement of the hypertrophic cartilage cells follows the same pattern as in the mesosternum; towards the posterior end there are two groups of concentrically arranged compressed cells situated side by side, indicating the dual origin of the ossification centre. The posterior half of the xiphisternum is still composed of unossified cartilage.

Stage 7. By the time of birth, the sternum has six well-ossified segments. Erosion has extended to both ends of each segment, and only thin terminal layers of very compressed cartilage cells remain. A number of bone trabeculae are present, forming a spongy tissue, the spaces of which contain marrow cells (Pl. 3, fig. 20).

At the sterno-costal junction, the cells have again undergone some rearrangement. Just before birth, after 19–20 days of gestation, the two regions of concentrically orientated cells next to each rib tip unite and nearly eliminate the central space which, as described above, was originally filled with loosely scattered cells. The chondroblasts then become rearranged in layers at right angles to the long axis of the sternum, suggesting an early stage in the formation of a transverse joint at the level of each pair of ribs (Pl. 3, fig. 20).

The xiphisternum has elongated relatively more than the other segments. Its posterior extremity, which remains cartilaginous, has expanded into a spatulate structure.

DISCUSSION

The histological study of the normal development of the mouse sternum furnishes further evidence to refute the theory of the costal origin of this structure. The early sternal rudiments are observed among two streams of cells migrating ventrally from the dorso-lateral body-wall. Though they originate just below the distal ends of the ribs, they arise quite independently of the latter, and the ribs themselves show no sign of any proliferation which might suggest that they participate in the formation of the sternum. It is true, however, that very soon after this initial stage, the ribs come into contact with the sternal rudiments and the two become intimately connected. As already suggested by Hanson (1919), this may account for the early workers' claim that the sternum has a costal origin.

Perhaps the most interesting and suggestive result of the study of normal sternal development is the observation that certain undifferentiated tissue elements in the dorso-lateral body-wall undergo changes together with the sternal rudiments. As described in §(*b*), the downgrowth of the sternal bands, and of other muscular and skeletal rudiments, is preceded by that of two streams of spindle-shaped mesenchymal cells. As the invading elements from either side approach each other, the original mesenchyme of the ventral body-wall degenerates and is replaced by downgrowing tissues. A further median degeneration occurs when the two streams of tissue meet in the mid-line. The degenerations of cells are correlated with the shrinkage of the ventral body-wall, as shown by the folding of the epidermis and the pericardium during this stage. Obviously the three changes, i.e. downgrowth, degeneration and contraction, are related, but it is not clear which of them is the primary factor. On the one hand, active downgrowth might cause the overcrowding of cells in the ventral body-wall and consequently produce degeneration and folding, but on the other hand if degeneration and contraction occurred spontaneously, they would tend to draw the dorso-lateral elements downwards. It seems more likely, however, that the downgrowth of the spindle-shaped cells plays a primary rôle, since it occurs before the median degeneration appears.

Very similar changes have also been described in the normal development of the avian sternum (Fell, 1939), and experiments suggested that in birds the downgrowth of the undifferentiated tissue may effect the closure of the sternum by pulling the two rudiments together. However, more than one factor may be concerned in the union of the sternal bars in the mammal; the possible effect of the elongation of the downward curving ribs, for instance, cannot be neglected. The mechanism of closure cannot be determined by the study of normal development alone and experimental work is required to settle this question.

Although it is clear that the ribs do not give rise to the sternal tissue and it is uncertain whether they influence the closure of the sternal rudiments, it seems likely that they may be concerned with the segmentation of the sternum. Segmentation is due to the persistence of small-celled cartilage at the sterno-costal junctions, which suggests that the ribs may inhibit the hypertrophy and ossification of the cells immediately next to them. As described above, at a very early stage the costal tissues are clearly demarcated from the sternal tissues; later the two tissues become intimately connected but later still they are once more sharply delimited. It is important to note the time relation between what happens in the sternum-rib association and what occurs in the sternal rudiments themselves. The intimate connexion between ribs and sternum is established immediately after the sternal rudiments in a given region have joined each other; at this stage there is morphological evidence that the rib tip presses hard into the sternal tissue so that it is difficult to distinguish between the two structures. The reappearance of delimitation and the restoration of the width of the sternal bands, which occur later, are apparently made possible by the growth of the sternal tissue in this region, as many mitoses can be observed there. It would seem that if any significance can be attached to this pressure of the ribs on the sternum, it ought to be connected with the segmentation of the latter rather than with the closure of the sternal bands, as it happens when the bands are already close together. On the other hand, it is not easy to visualize the means by which this short spell of pressure by the ribs could influence the cartilage cells at the sterno-costal junction so as to prevent their hypertrophy. Moreover, there is always the possibility that segmentation of the sternum is due to intrinsic factors and is independent of the ribs. This problem again cannot be solved by the study of the normal development only, and experimental work is necessary.

SUMMARY

The normal development of the mouse sternum was studied in embryos from 12 days of age and in infant mice 1 day old.

The sternum appears at about the twelfth day as a pair of mesenchymal condensations immediately below the clavicle and the ventral ends of the ribs. The two rudiments are widely separated and are situated on either side of the dorso-lateral body-wall.

The early rudiments are sharply demarcated from the clavicles and the ribs; there is no evidence that they are derived from either of these structures.

Preceding and accompanying the formation of sternal rudiments, there is a general downgrowth of tissues from the dorso-lateral body-wall, i.e. first of undifferentiated spindle-shaped cells and then of the differentiated tissues including the pectoral muscles, clavicles, ribs and sternal rudiments. This downgrowth which proceeds as a wave from the anterior part of the thorax posteriorly, pushes the pericardial cavity caudalwards and replaces the original mesenchyme of the ventral body-wall which degenerates. Where the two downgrowing streams from the dorso-lateral region meet in the mid-line, a median tract of further degeneration appears. This degeneration is correlated with a shrinkage of the mid-ventral body-wall as evidenced by the folded and thickened condition of the median ectoderm and pericardium.

From the twelfth day onwards, the sternal rudiments elongate and chondrify. With the downgrowth of the dorso-lateral tissues they move towards the mid-line and fuse with each other to form a cartilaginous rod. The fusion begins in the front and proceeds posteriorly.

The ribs come in intimate contact with the sternal tissue whenever fusion of the sternal rudiments is complete, but later a clear demarcation by fibrous tissue is again established between the ribs and the sternal tissue.

The anterior end of each sternal band grows out to form two lateral processes, the omosterna. The posterior end of the sternum elongates to form the xiphisternum and the tip becomes flattened and spade-shaped.

The middle portion becomes segmented. Segmentation of the sternum begins as a result of the hypertrophy of cartilage cells in the intercostal regions while small-celled cartilage persists at the sterno-costal junctions. Hypertrophy is followed by ossification which occurs in six centres with the formation of both endochondral and periosteal bone.

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EXPLANATION OF PLATES

Abbreviations

bv	blood vessel	*pst*	presternum
cl	clavicle	r_1	first rib
dc	degenerate cells	r_2	second rib
f	folding	r_3	third rib
h	heart	r_4	fourth rib
im	intercostal muscle	r_7	seventh rib
ost	omosternum	*sfc*	stream of flattened cells
pc	pericardial cavity	*st*	sternum
pme	pericardial membrane	*t*	trachea
pm	pectoral muscle	*xst*	xiphisternum

PLATE 1

Fig. 1. Transverse section of the thorax of a 12-day embryo at the level of the clavicle, before the appearance of the sternal rudiment. Haematoxylin and chromotrope. ×28.

Fig. 2*a*. Transverse section of the thorax of the same embryo as that of fig. 1 at the level of the first costal process. The stream of flattened cells are migrating just below the base of the limb bud. Haematoxylin and chromotrope. ×28.

Fig. 2*b*. Part of the same section to show the stream of flattened cells. ×90.

Fig. 3*a*. Transverse section of the thorax of a 12-day embryo, at the level of the clavicle. The two streams of flattened cells have nearly met. Necrotic cells are seen in the thoracic floor around the mid-line. Haematoxylin and chromotrope. ×28.

Fig. 3*b*. Part of the same section to show the degenerate cells. ×380.

Fig. 4. Transverse section of the thorax of the same embryo as that shown in fig. 3, at the level of the first rib. The rib is sharply demarcated from the streams of downward migrating spindle-shaped cells. Haematoxylin and chromotrope. ×28.

Fig. 5. Transverse section of an embryo at stage 1 (about 12½ days), at the level of the clavicle. The sternal rudiments are seen lateral to the distal end of the clavicle, in the dorsal part of the stream of the flattened cells. Haematoxylin and chromotrope. ×21.

Fig. 6. Transverse section of the same embryo, a few sections in front of that shown in fig. 5. The two streams of spindle-shaped cells form a continuous band below the clavicles. Degenerate cells are seen along the mid-line. The pericardial cavity is much reduced in this region. Haematoxylin and chromotrope. ×88.

Fig. 7. Transverse section of the thorax of the same embryo as that shown in fig. 5 just behind the clavicle. Note the necrotic pericardial membrane enclosed by the trough of flattened cells, and also the sharp demarcation between the sternal end of the rib and the sternal rudiment. Haematoxylin and chromotrope. ×45.

Fig. 8. Transverse section of the thorax of a 13½-day embryo (stage 3), at the level of the first rib. Note the radiating arrangement of the cells from the point of union of the muscles. An intimate connexion is now established between the first rib and the sternal tissue. The arrangement of the cells in the two tissues, however, is different. Haematoxylin and chromotrope. ×90.

PLATE 2

Fig. 9*a*. Transverse section of the thorax of the same embryo as that shown in fig. 8 at the level of the fourth sternal rib. The sternal rudiments are on the point of uniting. Note the folded epidermis and the degenerating cells in the mid-ventral body-wall. Azan. ×24.

Fig. 9*b*. Part of the same section to show the degenerate cells in the ventral body-wall and the folded epidermis. ×360.

Fig. 10. Frontal section of the thorax of a 13-day embryo (stage 2). The two mesenchymatous condensations of the sternal rudiments are widely separated. The ribs are clearly outlined. Haematoxylin and chromotrope. ×24.

Fig. 11. Transverse section of a 13-day embryo (stage 2), at the level of the clavicle. The sternal rudiments are seen lateral to the cartilaginous ends of the clavicles. Haematoxylin and chromotrope. ×110.

Fig. 12. Frontal section of the thorax of an embryo at stage 4 (about 14 days), to show the relation between the ribs and the sternal rudiments; the anterior end of the sternum is not seen in the section. Haematoxylin and chromotrope. ×33.

Fig. 13. Transverse section of a 14-day embryo (stage 4). The sternal rudiments have fused to form a broad cartilaginous band. The cartilaginous articulations of the clavicles are seen above the sternum. Haematoxylin and chromotrope. ×90.

Fig. 14. Transverse section of the thorax of the same embryo as fig. 13, at the level of the first sternal rib. The sternum has become wedge-shaped. Haematoxylin and chromotrope. ×90.

Fig. 15. Transverse section of the thorax of a 14-day embryo (stage 4), at the level of the sixth and seventh sternal ribs. Fusion of the sternal bands is not yet complete. Haematoxylin and chromotrope. ×100.

PLATE 3

Fig. 16*a*. Frontal section of the sternal rudiment of a 16-day embryo (stage 5). Signs of chondroblastic hypertrophy are seen in the first three and the last segments. Azan. ×25.

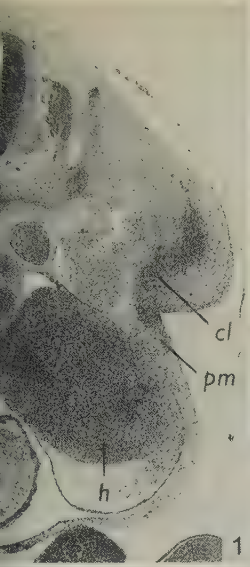
Fig. 16*b*. Part of the same section to show the slightly hypertrophic cells in the intercostal region. The sterno-costal junctions have increased in width; a layer of flattened chondroblasts marks the boundary between the sternum and the rib. Note the arrangement of the sternal cells into three groups between the two rib tips. × 96.

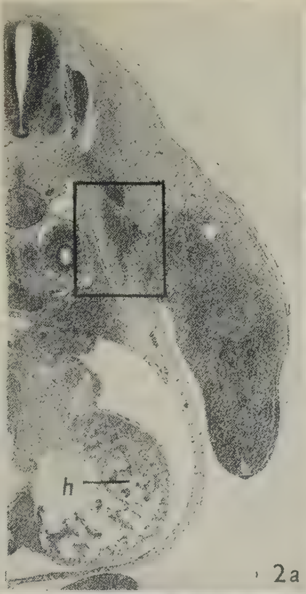
Fig. 17. Frontal section of the sternal rudiment of a 16-day embryo slightly more advanced than that shown in fig. 16. Only the first segment is shown. The first sign of ossification is seen in the two-layered structure of the periosteum. Haematoxylin and chromotrope. × 100.

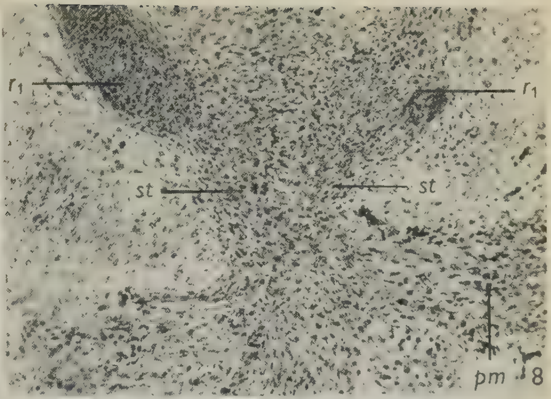
Fig. 18. Frontal section of the sternal rudiment of an 18-day embryo (stage 6). Only the first segment and the second sterno-costal junction are shown. Ossification is well advanced; a thick periosteal bone collar has formed; erosion has begun in the middle of the segment and the different zones of cells can be clearly seen. Azan. × 85.

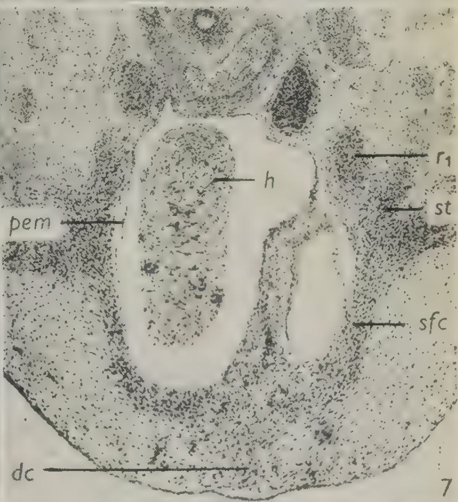
Fig. 19. Whole mount of the sternum from an 18-day embryo (stage 6) to show the elongated and broad xiphisternum. The anterior portion is eroded; the middle portion is hypertrophied and the cartilaginous posterior end is expanded into a spade shape. Dilute haematoxylin. × 30.

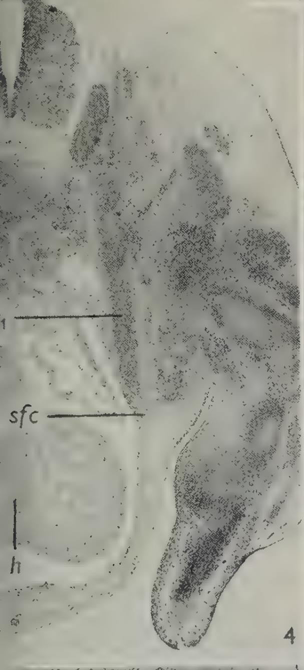
Fig. 20. Frontal section of the sternal rudiment at the time of birth (stage 7). Erosion has extended to the two ends of the segment; bone trabeculae have formed. Cells at the sterno-costal junctions have become rearranged into horizontal layers, suggesting the formation of transverse joints. Haematoxylin and chromotrope. × 48.

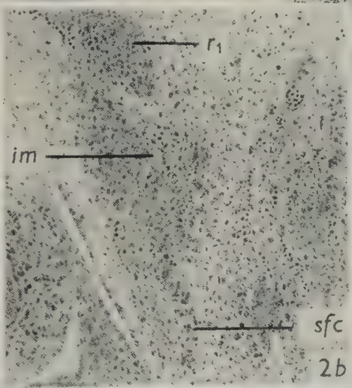


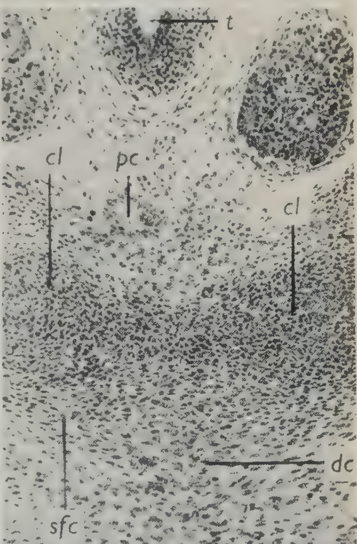


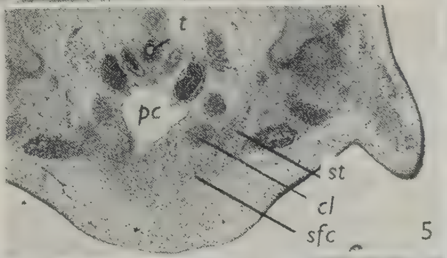


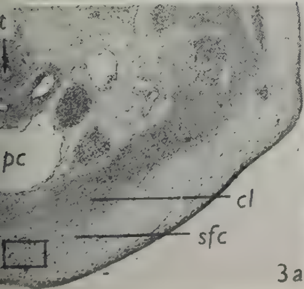


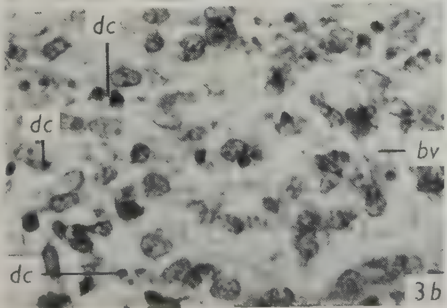












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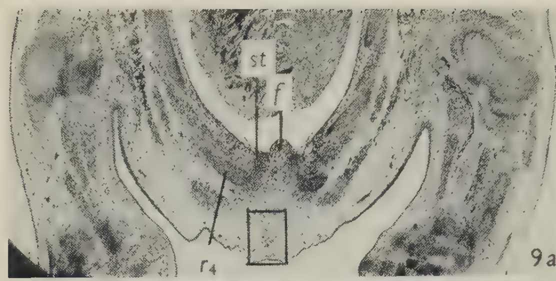

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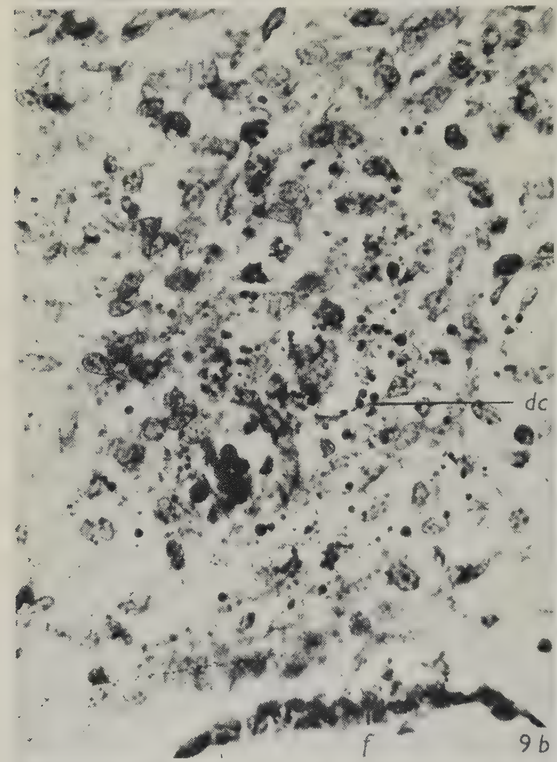

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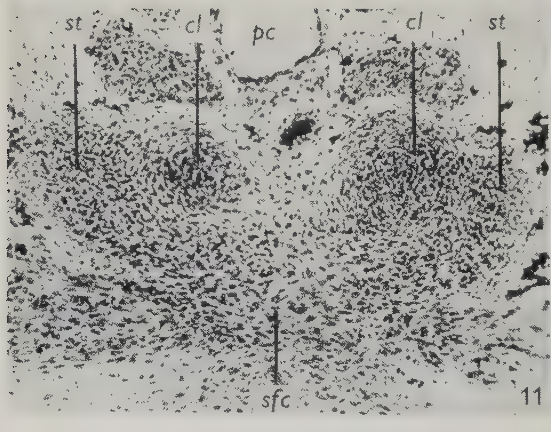

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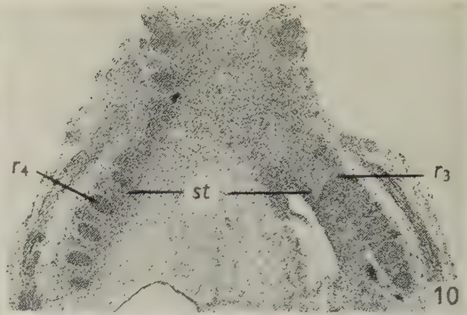

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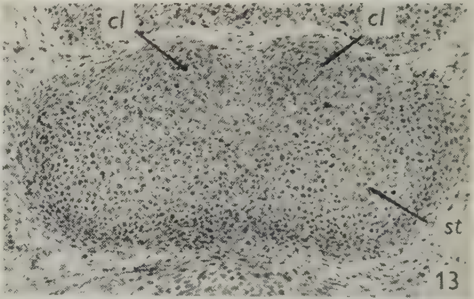

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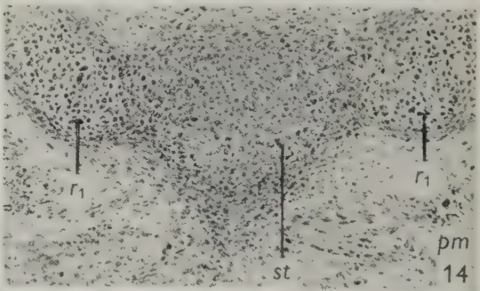

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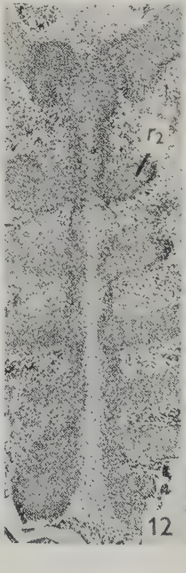

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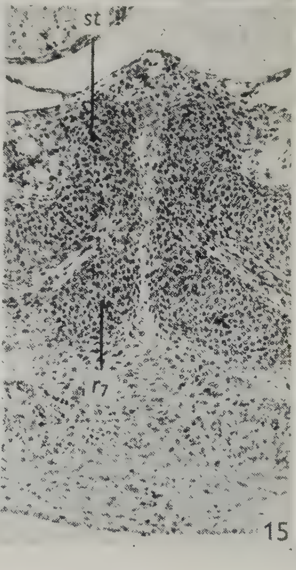

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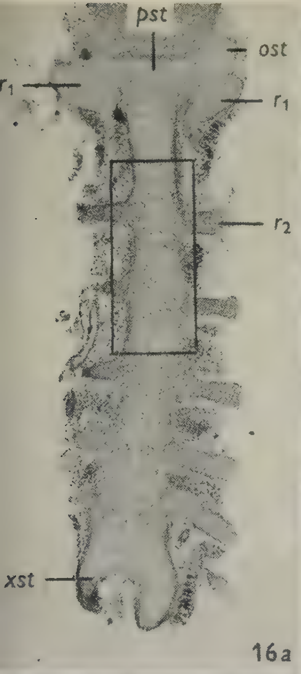

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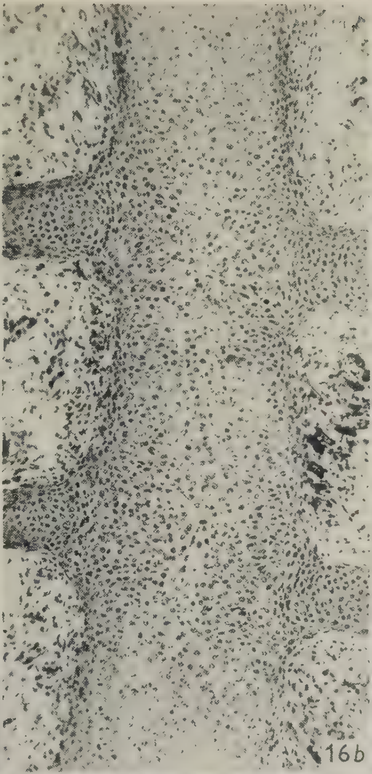

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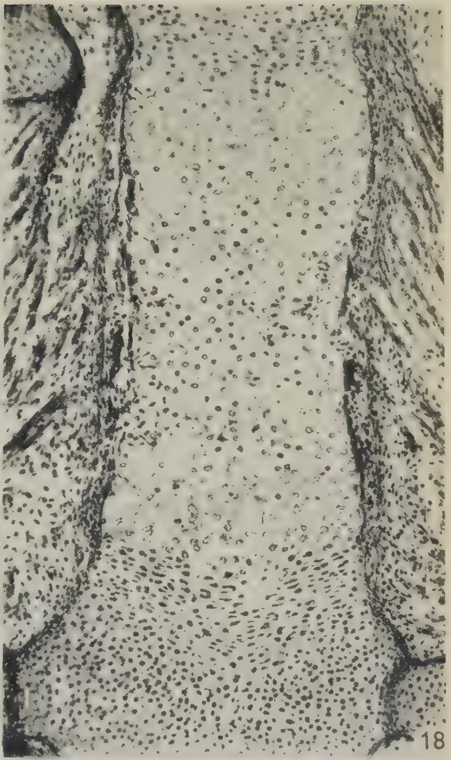

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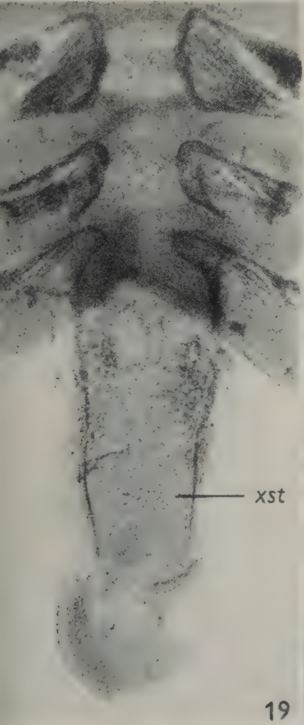

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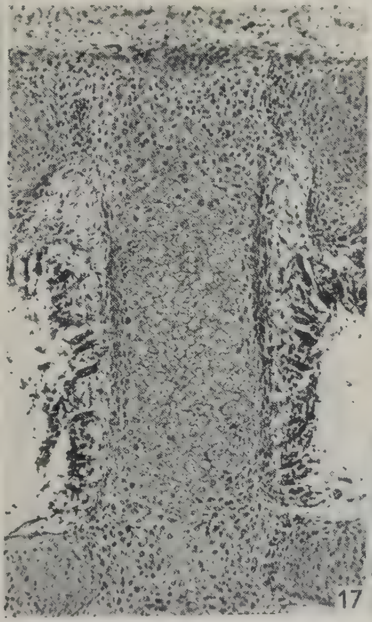

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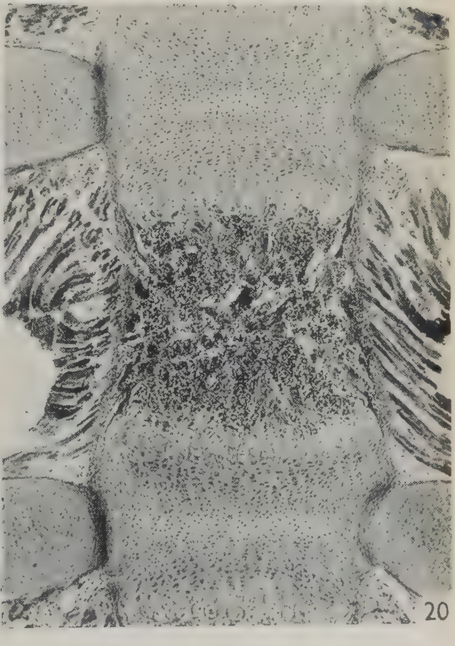

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JUI MING CHEN. I.—STUDIES ON THE MORPHOGENESIS OF THE MOUSE STERNUM

STUDIES ON THE MORPHOGENESIS OF THE MOUSE STERNUM

II. EXPERIMENTS ON THE ORIGIN OF THE STERNUM AND ITS CAPACITY FOR SELF-DIFFERENTIATION *IN VITRO*

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INTRODUCTION

The origin of the sternum has been as much disputed in mammals as in birds (Fell, 1939). Three theories have been put forward: one derives the sternum from the ventral ends of the ribs—the costal origin (Ruge, 1880; Mueller, 1906); another stresses its intimate relationship with the shoulder girdle—the coracoidal origin (Parker, 1891); and a third suggests that it arises independently of either ribs or coracoid—the '*in situ*' theory (Whitehead & Waddell, 1911). Detailed comparative and descriptive studies first by Hanson (1919) and later by Gladstone & Wakeley (1932) favour the '*in situ*' theory, as also do the observations recorded in Part I of the present series in which the normal embryonic development of the mouse sternum is described (Chen, 1952). So far, however, no experimental evidence such as that reported in birds (Fell, 1939) has been presented to demonstrate conclusively where the presumptive sternal tissue originates.

There has been a similar want of information on the capacity for self-differentiation of the mammalian sternum. Experiments have shown that the gross anatomical structure of the avian sternal rudiment is formed under the influence of a growth pattern intrinsic in the rudiment from a very early stage (Fell, 1939); it seemed interesting to discover how far the same is true of the mammalian sternum, the structure of which differs greatly from that of the avian sternum owing to its different functional requirements.

In the first section of the present study the tissue culture technique was used to investigate the origin of the mouse sternum. Fragments of the dorso-lateral body-wall from very young mouse embryos were cultivated *in vitro* with the two main objects: (*a*) to find whether the sternal bands can develop in the absence of the ribs, and (*b*) to define the approximate location of the presumptive sternal tissue and thus to ascertain whether the sternum should be regarded as part of the axial or of the appendicular skeleton.

The experiments described in the second section were made to find how far the development of the mouse sternum is regulated by intrinsic and how far by extrinsic agencies. Sternal rudiments removed from embryos of different ages were explanted *in vitro* and their behaviour during subsequent cultivation was studied.

TECHNIQUE

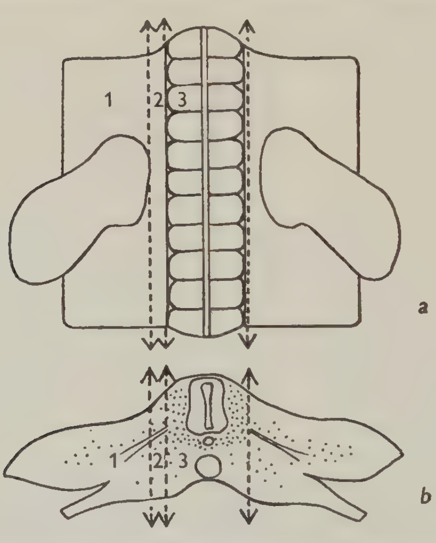
The tissue culture technique employed in this study was essentially the same as that described by Fell (1939), and both the hanging-drop and the watch-glass methods were used. Similar precautions were also taken to remove the ectoderm from tissue fragments to be cultivated so as to ensure the free access of nutrients to the tissue.

Camera lucida drawings of the living explants were made daily, and photographs also were taken occasionally. At the end of cultivation, some of the explants were fixed in 5% acetic alcohol, stained with carmalum or dilute haematoxylin and mounted whole. Others were fixed in 3% acetic Zenker's solution and were then serially sectioned in the usual way; the slides were stained with Ehrlich's haematoxylin and chromotrope 2R.

(1) THE ORIGIN OF THE STERNUM

Material and methods

Mouse embryos, 10–11½ days old, were used. The embryo was decapitated and the posterior end from just in front of the hind-limb buds was cut off. The remaining trunk portion was then opened and the viscera were removed. In group 1 (thirty-nine explants) the lateral body-wall with the fore-limb bud attached was excised as a strip from one or both sides (position 1, Text-fig. 1). In twenty-six experiments, the axial mesoderm, i.e. the somites and the perichordal tissue, was divided lengthwise and either the whole middle region comprising the medullary cord, the notochord and the somites was explanted (group 2, positions 2 and 3, Text-fig. 1), or the ventral half only of the somitic region (group 3, position 2, Text-fig. 1). In all three groups the axial mesoderm, when not explanted, was fixed and sectioned to ascertain the exact boundary of the tissue fragments removed for cultivation.



Text-fig. 1. Diagram of the operation for the preparation of the explants. The strips removed for cultivation are numbered in the same order as in the text. *a*, surface view; *b*, transverse section.

Most of the skin was dissected from the tissue before explantation, and the pieces that were left behind were removed after 2 or 3 days' growth when the explants were transferred to fresh medium. The cultures were maintained for another 3 or 4 days, and were then fixed and mounted whole.

Transverse sections of the embryos from which the body-wall had been removed for cultivation showed that the development was at a stage early enough for the purpose of the present experiment, since not the slightest sign of rib or sternal rudiments was present. They also showed that the parts removed for explantation were roughly of the same location as shown in the diagram. Thus in group 1, the lateral body-wall together with the limb-bud had been cut off a little below the ends of the somites; in group 2, the entire somitic region and the medullary cord had been amputated; and in group 3, the medullary cord was still intact but was quite bare of mesoderm on one side, since the somitic region had been removed.

Results

In twenty-nine of the thirty-nine explants in group 1, some part of the sternal band, together with clavicle, acromion, part of the scapula, the proximal, and sometimes also the distal, part of the limb-skeleton developed; no trace of ribs appeared in any of the explants. The percentage of success in these experiments was rather low, owing to technical difficulties which will be discussed later. The results are summarized in Table 1.

Table 1. *Development of skeletal rudiments in the explants of lateral body-wall with the fore limb-bud* (*group* 1)

	Appendicular skeleton				
No. of explants	**Humerus**	**Scapula**	**Clavicle**	**Sternum**	**Rib**
12	+	+	+	+	−
10	−	+	+	+	−
7	+	−	+	+	−
Total 29	19	22	29	29	0

(+ = present; − = absent.)
This table shows that in these experiments the sternal anlage is independent of the ribs.

The original explant appeared as a flat piece of tissue with the limb-bud protruding from one side; it was quite uniform in consistency with no obvious condensation. After 24 hr. growth, the outline of the humerus could be distinguished, followed by the condensation of the scapula and the more distal part of the early procartilaginous limb-skeleton which became quite distinct after the first passage to fresh medium. The clavicles, which appeared rather abruptly after 4 or 5 days, were already ossified when first identified. The rudiment of the sternal band could usually be seen after 6 or 7 days *in vitro*, as an oblong mesodermal thickening, at the lower end of the clavicle (Pl. 1, fig. 1). Further cultivation resulted in the chondrification of the sternal band the outline of which then became more distinct. In a few cultures the sternal band did not elongate much but appeared as a short rod, probably because part of the presumptive sternal tissue was lost during the dissection.

The explants of axial mesoderm differentiated *in vitro* to a certain extent, and ribs, segmental muscles and perichordal cartilage developed. Sternal bands never appeared, however.

The explants of group 2, which consisted of the axial mesoderm, usually formed a bar of cartilage beside the nerve cord, presumably representing the vertebral column. Its outline was often irregular and sometimes appeared to be broken up into nodules of cartilage instead of forming a continuous bar. In a few cultures, the perichordal sheath had chondrified, and was segmented by a series of constrictions (Pl. 1, fig. 2).

The explants of group 3, which comprised part of the somitic region, usually formed segmental muscles and ribs. The ribs were either packed abnormally close together or distorted, forming a row of cartilaginous nodules (Pl. 1, fig. 3).

(2) THE DEVELOPMENT *IN VITRO* OF THE ISOLATED MOUSE STERNUM

(*a*) *Cultivation of whole sternal rudiments*

These experiments were made to see how far sternal rudiments of different ages when isolated from the pectoral muscle, viscera, blood and nerve supply can differentiate normally.

Material and methods

Mouse embryos, 12 15 days old, were used. To prepare the explants, the embryo was decapitated and its hind-end cut off from just in front of the hind-leg buds. Most of the fore-limbs were removed, only a very short stub of humerus being left at the end of the clavicle. The trunk was then cut open dorsally along the spinal cord, and the viscera carefully removed so as to expose the lateral and ventral body-wall from the inside. Half of the costal region of either side was cut off and the remaining ventro-lateral body-wall laid with the skin surface upwards. The skin was then dissected away completely in the case of older embryos and partially in younger ones. The pectoral muscles were next removed, except for a few tags, and finally the remains of the clavicles and ribs were cut off near their articulation with the sternal bands, only short stumps being left attached.

During the first subcultivation, any skin not removed before explantation could be stripped off. Besides the routine removal of out-growing tissue at each subcultivation, it was sometimes necessary to dissect away traces of muscle and connective tissue to keep the sternal rudiment quite bare, which facilitates differentiation. The period of cultivation varied from 1 day to a month, depending on the age of the material and its degree of development.

Results

In view of the possibility that the age of the embryo may influence the degree of differentiation which the isolated sternum will undergo *in vitro*, a series of cultures were made from embryos of different ages.

Series 1: *explants from* $12\frac{1}{2}$-*day embryos*. A few experiments were made with rudiments from $12\frac{1}{2}$-day embryos, but the results were much less satisfactory than those obtained from slightly older material.

The sternal rudiments were a little younger than those of stage 2 in normal development (Chen, 1952). Their development *in vitro* was often somewhat abnormal. When first explanted, the sternal bands were very wide apart and consisted mainly of condensed mesenchymal cells. During cultivation they moved towards each other as *in vivo*, and after 4 days *in vitro*, were just in contact at the anterior end. Despite the fact that a number of mitoses could be seen here and there, there was very little, if any, growth in length of the sternal bands and the stumps of the ribs attached to them were packed together, leaving little space for intercostal regions (Pl. 1, fig. 4); in extreme cases, the last two or three pairs of ribs coalesced to form a cartilaginous plate attached to the sternum. The ribs could be seen pushing into the sternal rudiments so that each band had a wavy appearance.

By the sixth day *in vitro*, fusion was still incomplete and the sternum appeared as two very broad bands with a narrow gap between them. There was no sign of any growth in length, but the sternum had broadened so that the rib tips no longer indented the sternal tissue. In a few explants, the first indication of chondroblastic hypertrophy could be detected, not in the usual segmental position, but along the inner edge of each sternal band.

Two days later, in the better fused explants, a wedge-shaped area of hypertrophy with the narrow end at the front, was present in the middle of the roughly triangular sternal plate. Attached to the latter were seven pairs of ribs in close proximity to each other (Pl. 1, fig. 5). Necrosis usually set in after 8 or 9 days' cultivation. In one explant, which was kept for 11 days, the hypertrophy spread slightly but was kept within the central area leaving two strips of tissue along either side unaffected. Because of the unsatisfactory growth, no culture was kept beyond this stage.

Series 2: explants from 13-day embryos. Altogether forty-nine explants were cultivated in fifteen experiments; seven controls were fixed without cultivation to indicate the stage of development at the beginning of the experiment.

When first explanted, the sternal rudiments were at about the second stage of normal development (Chen, 1952) and could usually be distinguished as two rather translucent bands running antero-caudally at the distal ends of the ribs on either side of the ventro-lateral body-wall. They were farther apart posteriorly than anteriorly, so that when first dissected out from the body, the explant was trapezoid in shape, with a small amount of lateral body-wall containing the rib tips bordering the two sides. At the anterior end small fragments of the two clavicles remained just beneath the small, outward bending ends of the sternal rudiments. The area of degeneration in the ventral body-wall was quite widespread anteriorly, but posteriorly it was less intense and more restricted to the median line. Sections revealed that the sternal rudiment was not clearly defined, being still only a mesenchymal condensation and continuous medially with the spindle-shaped cells mentioned in Part I (Chen, 1952). The ribs, however, were clearly outlined.

The development *in vitro* of the sternal rudiments of this stage followed the normal sequence observed *in vivo*. The movement towards the mid-line and fusion of the two bars, segmental hypertrophy and even ossification could be observed in the cultivated rudiments. The only difference from the normal was that the rates of growth and of differentiation were retarded to a varying degree in different cultures. Some of the explants differentiated so slowly that they did not get beyond the stage of early hypertrophy before showing signs of necrosis. Fusion was not always complete at the hind-end, and a xiphisternum seldom developed. In a few cultures, however, development was most satisfactory; five hypertrophied segments differentiated, periosteal ossification took place and there was even a trace of the xiphisternal structure. Details of development *in vitro* are given below.

After 16–24 hr. cultivation, cells had migrated in bundles or sheets from the margins of the explants into the surrounding medium. This unorganized amoeboid outgrowth of cells from the explant is a familiar phenomenon in tissue culture and reappeared at every subcultivation. As a result of this cell migration, the tissue around the sternum became thinner and the explant less opaque, with all its elements more distinct. The sternal rudiments now appeared greyish by transmitted light and the rib tips a light yellowish colour. By thinning out the explant, the unorganized growth probably facilitated the supply of fresh food and oxygen to the sternum.

In the interior of the explant, the sternal bands had moved toward the median line so that their anterior ends were almost in contact. The bands had also increased in width but there was no noticeable increase in length. The ventral degeneration could still be seen as a greyish area in the median body-wall.

After 48 hr., the sternal bands were in contact anteriorly; farther back they had moved closer together and appeared almost parallel in most of the explants, though still fairly wide apart in others. Five explants were fixed at this stage, four for whole mounts and one for serial sections. Sections showed that the anterior portion of the sternal rudiments now consisted of procartilage instead of the mesenchymal condensation of which it was composed when first explanted.

By the end of the third day *in vitro*, the bands were in contact throughout almost their whole length. There was no actual fusion, however, except in the most anterior region. The colour of the sternal rudiments in the living culture appeared to have changed from grey to light yellow, somewhat like that of the ribs. This change of colour, as proved by the microscopic examination of sections, was a sign of chondrification. The ribs were in close contact with the sternum. Four explants were fixed, one for serial sections and three for whole mounts.

Transverse sections showed that the sternal rudiments now consisted mainly of round cells closely packed together with a little ground substance. They were identical with those found *in vivo* at about stage 3, and some were undergoing mitosis. There was a well-defined layer of perichondrium. Except in the most anterior portion, the double nature of the sternal rudiment was readily discernible. The ribs were penetrating into the sternal rudiment, but the two structures could still be distinguished from one another, mainly by the different arrangement of their cells (Pl. 1, fig. 6). In the whole mounts it was clear that these cultivated sterna were much wider than in the corresponding stage *in vivo*, especially towards the hind-end.

Degeneration in the mid-ventral body-wall was now restricted to a very narrow line.

Except those which were fixed, all the remaining thirty-seven explants were subcultivated after 72 hr. growth. Up to this time, i.e. before the first passage of explants to a fresh medium, no significant difference in the rate of differentiation could be noticed between different cultures, except that in some the sternal rudiments were closer together than in others. Variation increased from now onwards, however, and the explants fell into roughly two groups according to their rate of development.

In the better developed group, fusion of the sternum was usually complete to the extreme posterior end by the third or fourth day *in vitro*, though in two rudiments the hind-end remained bifid. The sternum became abnormally wide in proportion to its length. Hypertrophy usually appeared on the fifth day and followed the usual pattern, beginning anteriorly in the first segment and spreading to the succeeding segments. Sections showed that after 6 days' cultivation hypertrophy was well advanced in the first segment and involved about half of the cartilage tissue. A very thin bone collar was formed inside the two-layered periosteum. Hypertrophy could also be detected in the second, third and fourth segments, but periosteal bone-formation had not yet extended to these segments nor was the periosteum differentiated into two layers. No xiphisternum was present. An interesting feature of hypertrophy *in vitro* that could be observed in this group was the bilaterality of the process; thus in the second and third segments especially, two hypertrophic areas situated side by side were present in each segment (Pl. 1, fig. 7).

After about 11 days' cultivation, most of the sternal rudiments had begun to ossify. Periosteal bone-formation appeared first, followed by excavation and erosion of the calcified hypertrophic cartilage. In the living cultures, the bone collar gave the intercostal segments a swollen appearance, and the eroded area appeared as a brown mass against the yellowish background of hypertrophic cartilage. Sections of two explants fixed after 11 days' cultivation (Pl. 1, fig. 8) showed that the intercostal segments, especially the first one, had the same cell arrangement as in normal development, i.e. a central region of eroded cartilage, next to which was the calcified hypertrophic cartilage and the very compressed cartilage cells at either end of the segments. Both layers of the periosteum were well developed. The bone collar was fairly thick in the first three or four segments. The bilateral structure in the second and third segments persisted to this stage and was even more conspicuous in some explants, where one side was sometimes slightly more advanced than the other. Altogether, five segments were present. At the sterno-costal junction the sternal tissue was quite broad and clearly demarcated from the rib tips. The arrangement of smaller and denser cells next to each rib tip could easily be detected below the second segment.

In one explant only, a tiny process could be seen at the posterior end which represented the xiphisternum; the cartilage was hypertrophic and periosteal bone had formed.

In four explants, although ossification began fairly early, it did not proceed so rapidly as described above. Sections of such explants fixed after 20 days *in vitro*, showed that the ossification had not gone beyond the initial stage of cartilage erosion.

Three explants were cultivated until the twenty-eighth day. No further changes could be seen except that the outgrowth became very feeble.

In the less differentiated group, the sternal rudiments moved together more slowly than in the explants of the previous group and movement ceased after 5–7 days *in vitro*. Anteriorly, the sternal rudiment was abnormally wide, while posteriorly fusion took place to a varying extent, but in all except two of the explants the hind-ends remained more or less bifid. The sternum did not attain its full length, and no xiphisternum developed. Sections of two explants, fixed after 4 and 5 days' cultivation respectively, suggested that histologically there was the same sluggishness in differentiation.

The rate of differentiation and growth was retarded even more in later stages of cultivation. Rudiments were fixed and sectioned at intervals of 6–18 days of cultivation. Most of them were fixed because no progress had been observed for some time, and histological examination showed little change either in the degree of fusion or in the histological differentiation. In nine explants, hypertrophy began in the first segment, where it took several days to develop, and often did not spread to the following segments (Pl. 1, fig. 9). Thereafter either necrosis set in or the cartilage ossified.

Series 3*: explants from* 14*-day embryos.* Twenty-three explants were cultivated in eight sets of experiments; six controls were fixed. The rudiments used in this series were between stages 3 and 4 of normal development (Chen, 1952); the two sternal bands were close to each other, especially for the anterior two-thirds of their length, but diverged posteriorly. The ribs, particularly the second and third pairs, were in intimate contact with, and even penetrated into, the sternal tissue. The degeneration in the ventral body-wall had become greatly restricted and appeared as a greyish, median line between the two bands.

On the whole, these explants developed better *in vitro* than those in series 2, and the rate of growth and differentiation, though still below that *in vivo*, was relatively higher. The fusion of the sternal rudiments was more complete, hypertrophy and ossification proceeded normally and the xiphisternum was usually formed. There was still some variation between different cultures, but less than in series 2; the variation was probably due in part to slight differences in the age of the embryos from which the explants were taken.

All the sternal bands lengthened and fused completely in the first 24–48 hr. *in vitro*. The ribs by this time had become clearly demarcated from the sternal tissue (Pl. 2, fig. 10). In fourteen explants the first sign of chondroblastic hypertrophy was noticed on the second or third day, while in nine others, hypertrophy started on the fourth day. The histological picture was exactly the same as *in vivo*. Five or six segments were eventually formed and the bilateral origin of hypertrophy could be detected readily in the lower segments (Pl. 2, fig. 11). On further cultivation, excavation of the hypertrophied areas occurred on the ninth day in the older explants and on the twelfth day in younger material and rapidly extended to the following segments (Pl. 2, fig. 12).

The same unequal rate of development between the two sides of the same segment could be observed as in series 2, suggesting bilateral centres of hypertrophy and ossification.

In all except three explants, the xiphisternum was well developed though a little shorter than normal. In the three exceptions, even though fusion of the entire sternal rudiment was not complete, hypertrophy and ossification went on as usual in the separate portions.

Histological examination of explants cultivated for more than 11 days showed that all the segments were eroded by the fourteenth day. Further cultivation brought no significant progress.

Series 4*: explants from* 15*-day embryos.* For this series, twenty-one sternal rudiments were explanted in six different experiments. Six controls fixed at the beginning of cultivation showed that the material had reached stage 5 of development (Chen, 1952). Thus the closure of the sternal bands was already accomplished before explantation. Development *in vitro* involved only two steps, i.e. segmental hypertrophy and ossification, which took place in the explants in almost exactly the same manner as *in vivo*. However, various minor deviations from the normal were noticed in different cultures.

The rate of development was always slower than *in vivo*, especially during the later stages. Hypertrophy occurred after 1 day *in vitro* and had extended to the first four and the last segments by the third day; periosteal bone-formation was observed on the fourth day, and erosion of hypertrophied cartilage on the sixth (Pl. 2, fig. 13). Ossification had begun in every segment by the eighth day, when the explant corresponded roughly to stage 6 of normal development. Further cultivation did not bring about much advance; after 17 days *in vitro*, for instance, though the sternum showed quite a number of bone trabeculae in the first three segments and in the xiphisternum, the other segments were still in the early stage of erosion.

As in the preceding series, the bilateral origin of the ossification centres could be seen in these explants; it was more evident in the lower segments where cell hypertrophy was often a little more advanced on one side than on the other. The same lack of uniformity within each segment could also be observed in the formation of periosteal bone, so that the thickness of the periosteum and bone collar might vary in different places.

At the sterno-costal junctions, where at the beginning of cultivation the rib tips apppeared to push into the sternal tissue, the latter was seen to have regained its width with the usual grouping of cells next to each rib tip. No indication of joint-formation was observed upon further cultivation.

These experiments show a striking correlation between the stage of the sternal anlage at explantation and the degree of morphological and histological differentiation reached subsequently in tissue culture.

(*b*) *Cultivation of half of the sternal rudiment*

The object of these experiments was to see whether the sternal bands can develop independently of each other.

Material and methods

Embryos of 13 to 14 days were used. The ventro-lateral body-wall containing the sternal rudiments was dissected as described previously. A cut along the mid-line of the ventral body-wall was made to separate the explant into two equal halves, each consisting of half the presternum with the cartilaginous articulation of the clavicle, and the sternal band with seven rib stumps on one side and the ventral body-wall on the other. Since the sternal bands are still separated for most of their length in 13- to 14-day embryos, each explant possessed a considerable amount of ventral body-wall. This was usually removed by a cut close to the sternal band, so that only a narrow strip was left attached to the half sternum.

Sections and whole mounts of the explants were prepared in the same way as described above.

Experiments and results

Twelve experiments were performed with thirty-eight pairs of sternal rudiments. Most of the rudiments were obtained from 13-day embryos, but a few $12\frac{1}{2}$- and 14-day foetuses were also used. In general, the results agreed with those obtained with whole sternal rudiments, but development was slower and less complete.

The eight pairs of sternal rudiments from $12\frac{1}{2}$-day embryos grew well for about 9 days; they enlarged and became well chondrified during this period, but no hypertrophy of cartilage cells could be detected. It was difficult to tell whether each band moved in a median direction, as in cultures of the complete sternal rudiment. In a few explants, however, to which a relatively large amount of other tissues, e.g. clavicles, ribs and ventral body-wall, remained attached, there was evidence that a shift in the position of the sternal rudiment sometimes occurred. For instance, when a big piece of clavicle stump was present, its growth seemed to push the anterior end of the sternal band so that the axis of the latter shifted from a vertical to an oblique position during cultivation. In others, which had rather long rib tips, the sternal band became curved presumably because of the pressure exerted by the elongating ribs. Most explants were not kept beyond 12 days *in vitro* when they showed signs of degeneration.

In twenty-one pairs of explants from 13-day embryos, the single bands increased in size and chondrified as usual. The cartilage cells began to enlarge in the first segment on the fourth day of cultivation. Hypertrophy began along the median margin of the sternal tissue in the middle of the intercostal region and spread upwards and downwards and also across to the other side where the ribs were attached. The area of hypertrophy eventually assumed a triangular shape with the median margin as the base of the triangle and the apex wedged between the attachments of two consecutive rib tips. The cells of the sternal tissue around each rib tip remained small and were arranged in very compressed concentric layers as in normal development and in cultures of the whole sternum. On further cultivation, chondroblastic hypertrophy extended to the second and third segments in some explants; in others, hypertrophy developed very slowly and seldom passed beyond the second segment, when the growth of the culture became feeble and signs of degeneration were seen (Pl. 2, fig. 14).

In a few of the explants, which were maintained for longer periods (up to 18 days), the first segment ossified (Pl. 2, fig. 15). The formation of the two-layered periosteum was readily observed; it appeared first on the median margin of the sternal tissue and the bone collar was soon deposited within it. In one explant which was grown for 29 days before being fixed and sectioned, well-developed bone trabeculae were present in the first segment, though the following segments were still cartilaginous without even cell hypertrophy.

All the nine pairs of explants from 14-day embryos differentiated better than those from the younger foetuses. Hypertrophy began on the second day and extended to the following segments fairly quickly; four segments could be detected after 6 days of cultivation. The area of hypertrophy was of the same triangular shape.

It is thus seen that half sterna can develop in culture, though the degree of morphological and histological differentiation reached is not as high as that of whole sterna explanted at the same stage.

DISCUSSION

The cultivation *in vitro* of isolated fragments of the lateral body-wall from very young embryos demonstrated that the sternum can develop in the complete absence of ribs. Furthermore, in explants of axial mesoderm, ribs were invariably formed but showed no evidence of giving rise to any sternal tissue. In explants of the lateral body-wall and limb-bud, a sternal band developed in only about 75 % of the cultures, but, as mentioned above, the negative results were probably due to technical reasons. Since the material used was at an early stage of development, the tissue fragments to be handled were small, thin and easily damaged. Also very little anatomical differentiation was discernible, so that it was not easy to dissect the explants accurately and without injury, while the necessity of removing the ectoderm added further difficulties. The sternal band may have failed to appear either because the presumptive sternal tissue was erroneously left out or because it was hopelessly damaged when the ectoderm was dissected away. On the other hand, many cultures were unsuccessful because the ectoderm was not completely removed and the enveloped tissue degenerated early in cultivation. Nevertheless, the more successful cultures indicated clearly that the sternal bands arise independently of the ribs and thus confirmed the conclusion previously drawn from the study of the normal development of the mouse sternum (Chen, 1952). These results agree with the earlier work on avian material (Fell, 1939). The fact that the costal processes developed only in explants of the axial mesoderm and especially in the somitic region, whereas the sternal bands appeared only in explants of the lateral body-wall close to the limb-bud, indicated that the presumptive rib tissue is located in the somitic region while the site of the sternal tissue is further lateral. Although no attempt was made in the present study to define the exact site of the presumptive sternal tissue, it was fairly clear from the results reported that it must be closely associated with the appendicular skeleton, since it always appeared immediately below the clavicle. However, it seems unlikely that the sternum is actually derived from the clavicle, for some explants formed a well-shaped clavicle without any trace of the sternal tissue. These observations agree both with Hommes's conclusion (1921) and with the results of Fell (1939) which showed that in the bird the sternal rudiments originate independently not only of the ribs but also of the clavicle.

The cultivation of sternal rudiments *in vitro* revealed their remarkable capacity for self-differentiation. The explanted sternal bands moved toward each other, fused to a varying extent, underwent segmental hypertrophy and became ossified as *in vivo*. It was evident that the development, like that of other skeletal structures, is largely controlled by intrinsic factors.

The importance of a suitable environment for the proper expression of this potency for self-differentiation was, however, equally evident from the experiments reported above. Whilst in the most satisfactory cultures development *in vitro* proceeded almost as *in vivo*, in the majority of the explants there were certain abnormalities. In the first place, the rate of development was retarded *in vitro*. Table 2 gives the time required for the rudiments *in vitro* to reach a corresponding stage *in vivo*. It is clear that the earlier the age at which the rudiments were explanted the greater was the retardation of development. The results indicated that the conditions *in vitro* are less favourable than *in vivo* and that younger rudiments were more susceptible to any change in environmental conditions than older ones.

Secondly, it was often found that *in vitro* succeeding stages in development overlapped each other. Thus when the closure of the sternal bars was delayed, the rudiment often proceeded to the next step, viz. chondrification and subsequent hypertrophy, before fusion of the bars was complete; chondroblastic hypertrophy in turn might not have extended to all the segments before ossification began in the first segment. It appeared that the rudiment was so determined for the various stages in development, that it could pass through each stage without requiring the completion of the previous step.

Table 2. *Time required for sternal rudiments* in vitro *to reach a corresponding stage* in vivo (*in days*)

Stage of development	Complete fusion of sternal bands	Chondroblastic hypertrophy	Ossification
	In vivo		
Approximate age of embryos (days)	15	16	17
	In vitro		
Explants from embryos of:			
12½ days	6*	8†	—
13 days	5–7‡	11*	16†
	3–4	6‡	11*
14 days	1–2	4–5	9–12
15 days	0	2–3	6–8

* Incomplete. † Slight. ‡ Not in the posterior end.

The delicate co-ordination normally present *in vivo* between these various developmental processes was disturbed by the retardation of growth and differentiation produced by conditions *in vitro*, with the result that certain structural abnormalities appeared in the explants. The most obvious example was the incomplete fusion of the sternal bands; this was probably due to the fact that the movement of the rudiments toward each other was so delayed that chondrification set in before they came into contact, and the well-formed perichondrium naturally rendered fusion difficult. Other structural defects in the explants, e.g. their abnormal width and diminished length, the absence of a xiphisternum and the bilateral appearance of the hypertrophic centres were probably due, at least in part, to the same fundamental cause. In short, the results agree with the statement that 'the full perfection even of the cartilage model cannot be achieved by the action of the intrinsic pattern of growth alone' (Murray, 1936).

The extreme case of disturbance in the normal balance of growth and differentiation, i.e. that of the development *in vitro* of very young rudiments, perhaps deserves more attention. It is of particular interest in that the resulting structure resembled the sternum of the screw-tail mouse in its early stage of embryonic development (Bryson, 1945). In the explants there was the same abnormal width, diminished length and lack of segmentation which characterized the sternum of this mutant. Although the fundamental cause for abnormal width and length may be different in each case, there is a strong suggestion that in both the lack of segmentation was related to the fact that the ribs were packed together, thus leaving no space for intercostal segments to appear. These observations suggest that probably segmentation is not inherent in the sternal rudiment, but, as held by Gladstone & Wakeley (1932), is a secondary phenomenon. This question will be considered in Part III of the present series of publications.

The results of cultivating half of the sternal rudiment were not wholly satisfactory, but they showed that a single sternal bar can develop independently in response to factors intrinsic in itself. The half rudiments were capable of chondrification, chondroblastic hypertrophy and ossification, and appeared to have as great a capacity for self-differentiation as the entire rudiment.

A single sternal band, however, was more affected by changes in environment than the complete rudiment. For one thing, the bilateral balance that usually existed with paired primordia was lost, which at least partly explains why the elongation of clavicles and ribs sometimes seriously distorted the shape and orientation of the half rudiment. Moreover, any disturbance which might be caused during the dissection of the explants was bound to be greater in isolating a single sternal band. As in the intact rudiment cultivated *in vitro*, the rate of development of the single halves was abnormally slow, the succeeding stages of development often overlapped and older rudiments differentiated *in vitro* better than young primordia.

SUMMARY

The origin of the sternum was studied experimentally by cultivating *in vitro* fragments of the undifferentiated body-wall isolated from 10- to 11½-day mouse embryos, to determine their developmental potencies.

The lateral body-wall of the fore limb-bud region, when removed before the appearance of the costal processes, formed a sternal rudiment and most of the appendicular skeleton but no ribs.

Explants of the axial region from the same embryos formed cartilage showing various degrees of segmentation; ribs developed in some cultures but were never associated with any sternal rudiments.

The results showed that sternal rudiments are not derived from the ribs.

The location of the presumptive tissue of the sternum suggested that it is closely associated with the skeleton of the fore-limb but is not derived from the clavicle.

The ventro-lateral body-wall, including both sternal bands, was removed from mouse embryos of different ages; it was dissected free of skin, pectoral muscles, most of the clavicles and all but the sternal ends of the ribs, and cultivated *in vitro*.

During cultivation, the sternal rudiments moved together and fused in the mid-line. Segmental hypertrophy occurred; ossification centres appeared, and a periosteal bone collar was formed.

Development and growth of the sternum were subnormal, but histological differentiation was much less retarded than growth.

Successive steps of development, e.g. chondrification, fusion of the two sternal bands, segmental hypertrophy and ossification, which normally begin at the anterior end of the rudiment and proceed posteriorly, appeared in normal sequence, but the

various steps were not completed at the posterior end so that the anatomical development was not always satisfactory. Only in one case was a normal xiphisternum formed.

In general, the older rudiments developed better than those from very young embryos.

Explants of half of the ventro-lateral body-wall containing only one sternal band and associated rib stumps were also cultivated. Each isolated sternal band developed segmental centres of hypertrophy and ossification but anatomical development was slower and less complete than that of whole sternum.

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EXPLANATION OF PLATES

Abbreviations

cl	clavicle	r_2	second rib
er	erosion	*ra*	radius
hc	hypertrophic cartilage	*sc*	scapula
hu	humerus	*st*	sternum
nc	nerve cord	*ul*	ulna
p	periosteum	*vc*	vertebral column
r	rib	*xst*	xiphisternum
r_1	first rib		

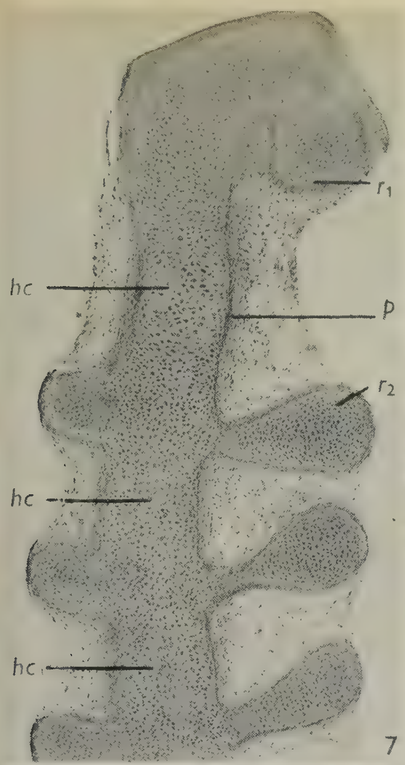
PLATE 1

Fig. 1. Whole mount of the lateral body-wall with the fore limb-bud (group 1) from an 11½-day embryo, cultivated *in vitro* for 7 days. The appendicular skeleton, the clavicle and the sternal rudiment have been formed. Dilute haematoxylin. × 22. Outline of the different elements is shown in the accompanying diagram (1*a*).

Fig. 2. Whole mount of an explant of the axial mesoderm (group 2) from the same embryo as fig. 1, cultivated *in vitro* for 7 days. Part of the vertebral column with ribs has developed. Carmalum. × 22.

Fig. 3. Whole mount of an explant of the somitic region (group 3) from an 11½-day embryo. Nodules of cartilage representing ribs are seen. Carmalum. × 22.

Fig. 4. Frontal section of a sternal rudiment from a 12½-day embryo after 4 days *in vitro*. The two sternal bands are short and are fused only at the anterior end. The ribs are packed together. Haematoxylin and chromotrope. × 32.



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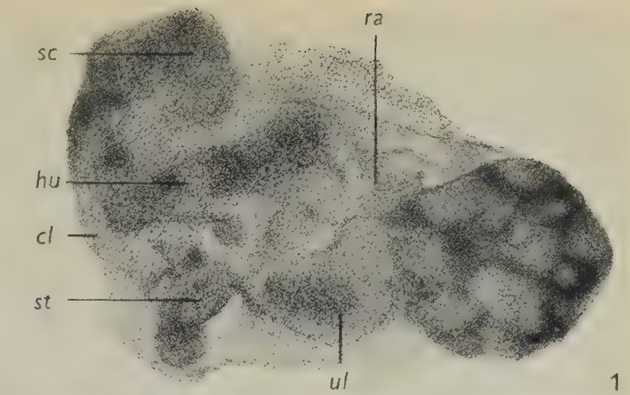

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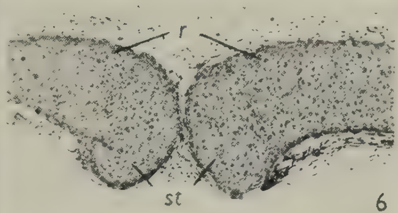

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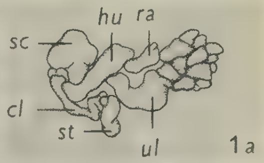

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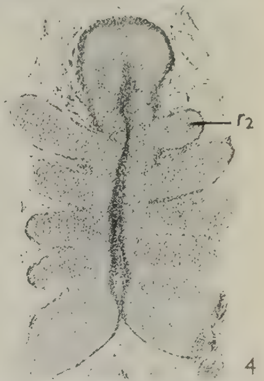

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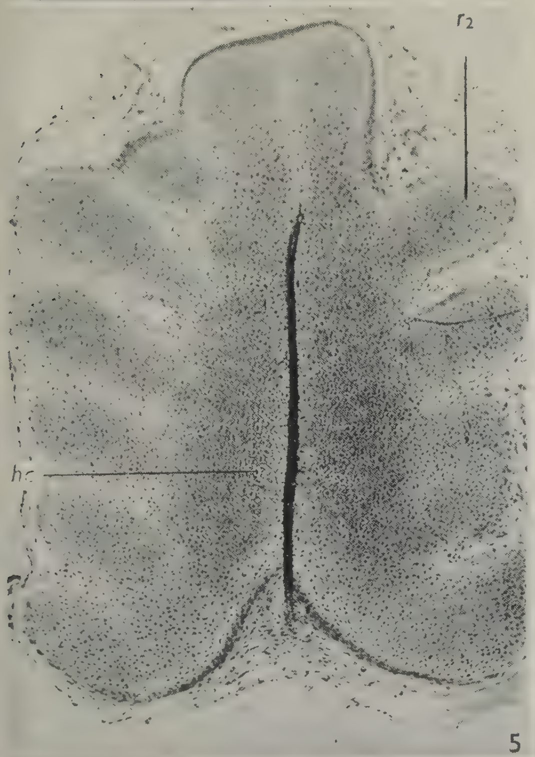

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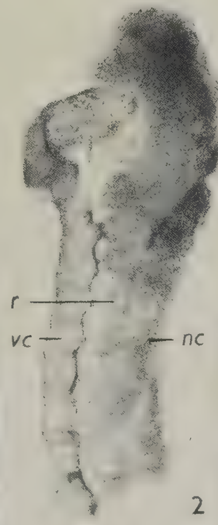

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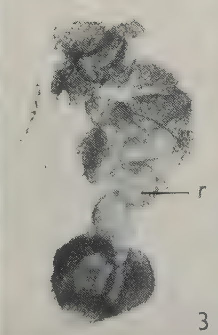

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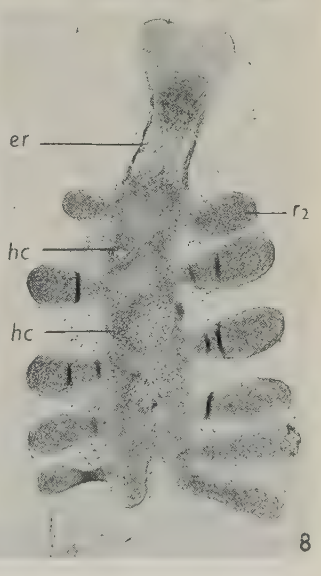

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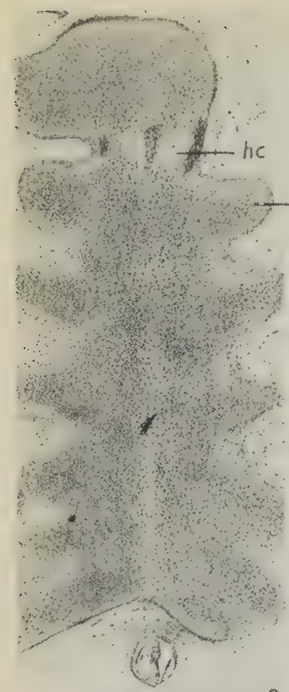
JUI MING CHEN. II—STUDIES ON THE MORPHOGENESIS OF THE MOUSE STERNUM



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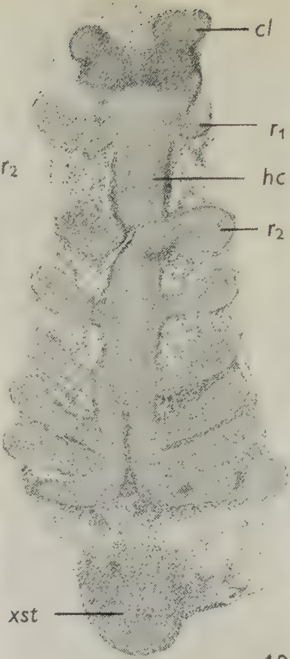

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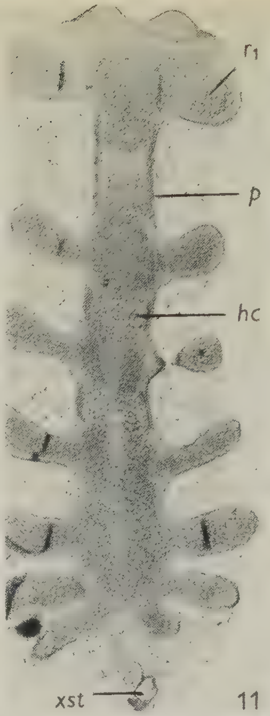

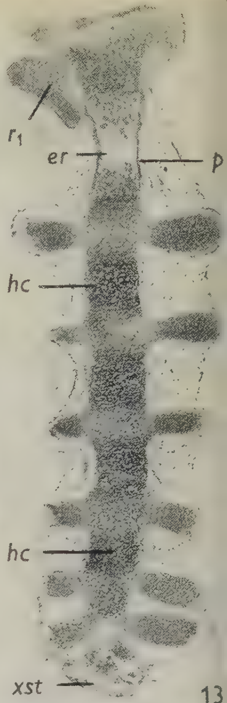

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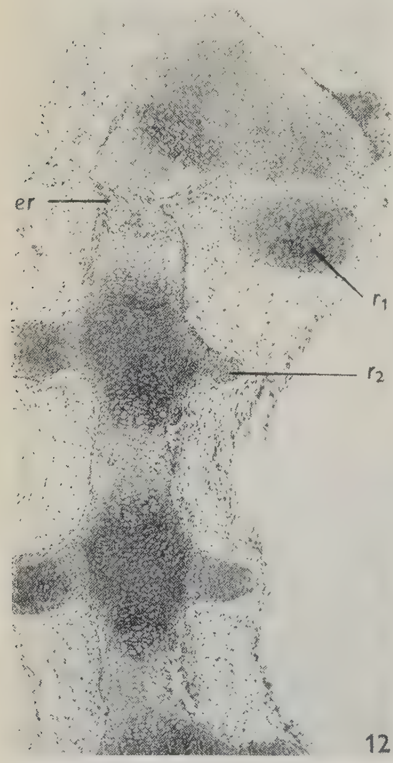

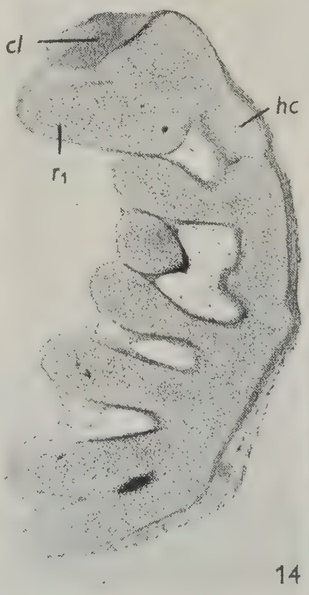

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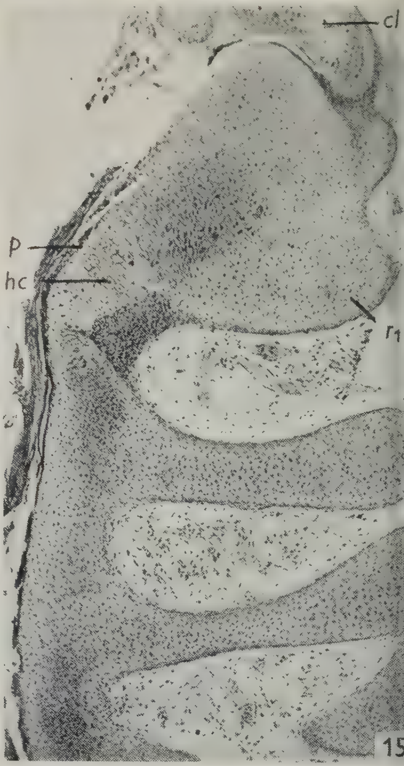

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JUI MING CHEN. II STUDIES ON THE MORPHOGENESIS OF THE MOUSE STERNUM

Fig. 5. Frontal section of a sternal rudiment from a 12½-day embryo after 8 days *in vitro*. The sternum has hardly increased in length but is very wide. Hypertrophic cartilage cells can be seen in the centre of the sternum. No segmentation is visible. (The line in the middle is an artefact due to the folding of the section.) Haematoxylin and chromotrope. × 58.

Fig. 6. Transverse section of a sternal rudiment from a 13½-day embryo after 3 days *in vitro*. The fusion of the two bands which consist of early cartilage cells is not complete. Note the intimate connexion between the sternum and the rib tissue; though the boundary between the two tissues is still distinguishable. Haematoxylin and chromotrope. × 90.

Fig. 7. Frontal section of a sternal rudiment from a 13-day embryo after 6 days *in vitro*. Chondroblastic hypertrophy is advanced in the first segment where the 2-layered structure of the periosteum is clearly shown and a periosteal bone collar has been formed. Hypertrophy is also seen in other segments. Haematoxylin and chromotrope. × 52.

Fig. 8. Frontal section of a sternal rudiment from a 13-day embryo after 11 days *in vitro*. The hypertrophic cartilage is eroded in the first segment where the cells are arranged as *in vivo*. Bilaterality of hypertrophy is clearly shown. Haematoxylin and chromotrope. × 30.

PLATE 2

Fig. 9. Frontal section of a sternal rudiment from a 13-day embryo after 11 days *in vitro*. Chondroblastic hypertrophy has begun in the intercostal regions and large vacuolated cells can be seen. Haematoxylin and chromotrope. × 32.

Fig. 10. Frontal section of a sternal rudiment from a 14-day embryo after 2 days *in vitro*. The sternum is well chondrified. The fusion is nearly as good as *in vivo* and a xiphisternum is seen. Chondroblastic hypertrophy has occurred in the first segment. Haematoxylin and chromotrope. × 28.

Fig. 11. Frontal section of the sternal rudiment from a 14-day embryo after 5 days' cultivation. Chondroblastic hypertrophy is well advanced in the first four segments and in the xiphisternum, only part of which is shown in this section. The two-layered structure of the periosteum is seen in the first three segments. Note that the bilateral origin of hypertrophy can still be distinguished in the third segment. Haematoxylin and chromotrope. × 22.

Fig. 12. Frontal section of the sternal rudiment from a 14-day embryo after 12 days' cultivation. The first three segments are seen. The erosion is similar to that *in vivo*. Haematoxylin and chromotrope. × 47.

Fig. 13. Frontal section of a sternal rudiment from a 15-day embryo after 6 days *in vitro*. Erosion of cartilage cells has just begun in the first segment where periosteal bone is also formed. Chondroblastic hypertrophy is seen in the other segments. Only part of the xiphisternum is shown in the section. Haematoxylin and chromotrope. × 20.

Fig. 14. Frontal section of a single sternal band from a 13-day embryo after 10 days *in vitro*. Chondroblastic hypertrophy is most advanced in the first segment. Haematoxylin and chromotrope. × 30.

Fig. 15. Frontal section of a single sternal band from a 13-day embryo after 18 days *in vitro*. Periosteal bone formation is seen in the first segment. Haematoxylin and chromotrope. × 58.

THE RELATIONSHIP OF CUTANEOUS SENSIBILITY TO NEUROHISTOLOGY IN THE HUMAN PINNA

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INTRODUCTION

In a recent investigation, Weddell & Zander (1950) compared and contrasted the histological methods commonly adopted for the display of neural elements in peripheral tissues, using the cornea as their testing ground and controlling their observations by phase-contrast examination of fresh untreated specimens. They found that the appearances produced depended to a significant degree on the precise technique adopted, and that no single method was of universal applicability. However, it was possible, by using the pictures obtained by silver impregnation and by methylene-blue staining to complement one another, to interpret the results in the light of the phase-contrast observations.

It appeared worth while to apply this newly gained experience to the examination of preparations of human skin. The area selected was the auricle of the ear, since in this region relatively large areas of thin skin are available, resting on a minimal base of relatively homogeneous subcutaneous tissue. At the same time, the sensitivity of the auricle to the four commonly recognized modalities of sensation was assessed in terms of the sensitivity of other skin areas.

There is surprisingly little precedent for an attempt to compare the sensory capacities of two areas of skin; the most recent paper is that of Hutchison, Tough & Wyburn (1948). The two control areas for the present investigation were the front of the forearm and the palmar aspect of a finger. The first of these was chosen since it has been the site of election for sensory experiments in the past, and has thus acquired the status of a standard area. The second was taken as representative of a specialized sensory region.

MATERIAL AND METHODS

A. *Sensory testing*

An investigation was made of the responses of a number of healthy men to stimulation of the cranial surface of the auricle by a variety of agents; no subject had previous experience of experiments involving sensory testing. In essentials the methods used were merely a refinement of the usual clinical methods. Since, however, there is evidence (Sinclair & Hinshaw, 1951*a*) that testing methods are an important factor in the results of sensory experiments of this kind, full details are given.

Comparisons between auricle and forearm

A testing area of 1 sq. cm. was marked out on the flattest available region by means of a square rubber stamp ruled in millimetres, care being taken to avoid any large veins. An exactly similar control area was established on the front of the right forearm 12 cm. above the proximal crease of the wrist.

In ten subjects each area was stimulated ten times at random by a vertically applied no. 2 nylon thread 2·5 cm. in length (Sinclair & Hinshaw, 1951 *b*): the subject was asked to shut his eyes and to respond every time he felt himself touched. The numbers of responses in the two areas were then compared. In the same ten subjects four or five pricks were then applied to each area with a long round-bodied needle (Sinclair & Hinshaw, 1951 *b*): the pricks were of a severity just short of drawing blood. The subject was then asked if he could detect any difference between the experiences produced in the two areas.

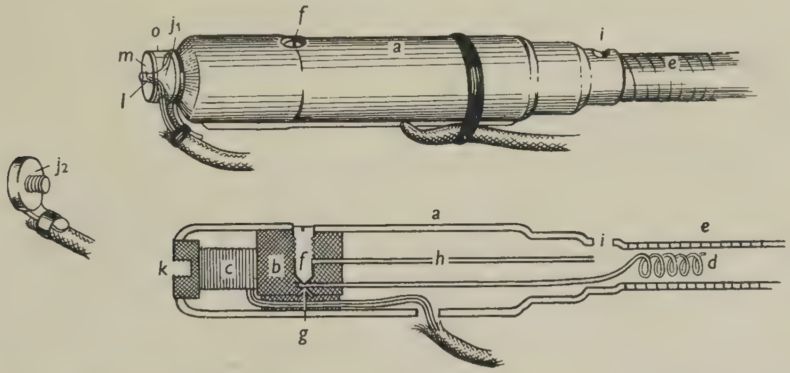

Fig. 1. *Temperature probe.* The vulcanite holder *a* contains a copper block *b* which can be heated by the coil *c* or cooled by allowing carbon dioxide under pressure to expand inside it. The gas is brought from the cylinder along the coiled stainless steel tube *d* which is enclosed in flexible gas piping *e* to allow the instrument mobility. The terminal part of the tube is straight and is let into the block *b*, where it is pierced at right angles by a small hole closed by the adjustable bevelled screw *f*. When this screw is partly withdrawn the gas rapidly expands at the point *g* and escapes past the screw to the waste pipe *h*, which leads it to the vent *i*. Copper tips of various shapes (j_1, j_2) can be screwed into the socket *k* and so heated or cooled. Each terminal bears a fine thermocouple *l* as close as possible to its tip. The small terminal j_1 which has a tip 1·5 mm. in diameter, is shielded by a perforated mica disc *m* which allows the tip to protrude 1 mm. beyond it and is secured to the base of the terminal by cellotape *o*; this mica reduces radiation to the skin from the base-plate. The large terminal j_2 (12·5 mm. in diameter) needs no shielding, since its whole surface is employed.

Temperature stimuli were applied to each area by means of a special probe which could be heated electrically or cooled by allowing carbon dioxide to expand through a small orifice in its interior (Fig. 1). This probe was fitted with a series of detachable copper tips, each carrying a thermocouple which recorded the temperature of the tip correct to within half a degree Centigrade. Ten subjects were used for the warm comparisons, and ten for the cold comparisons.

In the first test 100 stimuli were given to the marked area, using a tip 1·5 mm. in diameter at a temperature of 45° C. This tip projected 1 mm. beyond a mica disc 12·5 mm. in diameter (Fig. 1) which served to prevent stimulation of the skin by radiation from the hot metal block. The stimuli were delivered in groups of ten, using the columns on the ruled area as a guide. The odd-numbered columns were covered first, then the even-numbered ones, with an interval of 1 min. between each column. Each stimulus remained in contact with the skin for 2 sec., and the time taken to complete one column was between 40 and 50 sec. The instrument was allowed to rest by its own weight on the forearm, but in the ear it was necessary to hold out the auricle at an angle to the side of the head and apply the stimulus at as constant a pressure as possible with the other hand.

The subject was aware that the stimulus was warm, but was also told that he might not experience anything beyond the feeling of the pressure of the instrument; he was asked to report immediately any sensation of warmth. The time available for each judgement was deliberately made short in view of the inexperience of the subjects; they were not asked to make any introspective evaluations.

After a rest of some 5 min. the control area was covered in exactly the same way and the numbers of responses in the two areas were subsequently compared. In order to neutralize any possible 'learning effect' the control area was taken first in five subjects and the experimental area first in the other five. Each man was asked if he observed any subjective difference between the sensations he had experienced in the two areas.

After a further rest of 5 min., a new area of skin in both ear and forearm was selected and an attempt was made to establish the threshold for the sensation of warmth, using this time a probe tip with a diameter of 12·5 mm. The threshold was approached from both directions in steps of 1° C., and the tip was allowed to remain in contact with the skin for a period of 10 sec. if no immediate response was obtained. As few stimuli as possible were applied in order to avoid the effect of fatigue, though it was found that with a stimulus of this size fatigue to a constant temperature did not occur until much later than the time taken by the threshold experiments. The threshold was taken as the mean of the reading above which every stimulus was reported as warm and the reading below which every stimulus was reported as neutral.

The cold comparisons in the remaining ten subjects were made in an exactly similar manner, the number of cold responses per 100 stimuli being obtained with the small tip of the instrument cooled to 15° C., and the threshold to cold being determined in the same way as the threshold to warm.

Comparisons between auricle and finger

Another thirty comparisons were made between the auricle and a control area sited on the front of the proximal phalanx of the right ring finger. The subjects used were for the most part those who took part in the forearm comparisons, and the only procedural difference was that in the touch comparisons a no. 1 nylon thread was used, since it was found that no. 2 thread gave 100 % responses from both testing areas.

'*Cold spots*'

In five subjects a number of areas especially sensitive to cold stimuli were located by a metal tip 1 mm. in diameter at a temperature of 15° C. When the same point on the skin had given rise to three successive and unequivocal responses of cold it was marked with a spot of indian ink carried into the skin on the point of a fine needle. The histology of these areas was studied after biopsy.

B. *Histology*

Pieces of skin from the region of the concha and antihelix of the cranial aspect of nineteen human ears were impregnated with silver by the method described by Weddell & Zander (1950) for the cornea. Minor *ad hoc* modifications were occasionally made to ensure that the axis cylinders of the nerve fibres in the skin were outlined selectively. Each piece of skin was between 1 and 2 sq.cm. in area, and in many of the ears several such pieces were studied; the total area of skin available was thus over 30 sq.cm. Three of the pieces contained marked spots which had previously been found to be sensitive to cold. In addition, three specimens of skin from the lateral aspect of the auricle and three portions of auricular cartilage were examined by the same technique. All material was taken so as to avoid the regions of the intrinsic muscles of the auricle.

Control sections were taken from the palmar aspect of an amputated human ring finger to act as a check on the silver impregnation technique.

Lastly, two specimens of skin from the cranial aspect of the auricle were stained by methylene blue in the living subject, after the location and marking of an area especially sensitive to cold. The dye was made up to a concentration of 0·02 % in physiological saline; it was injected intradermally, and the skin was removed 25 min. after injection. The dye was fixed and the skin prepared for microscopic examination with due regard to the various precautions listed by Weddell & Zander (1950).

OBSERVATIONS

A. *Sensory testing*

Comparison of auricle with forearm

(1) *Touch.* Every stimulus on every ear was appreciated, but only 68 % of the stimuli on the forearm were felt; the difference is statistically significant ($p < 0·01$).

(2) *Pinprick.* Six subjects thought the ear pricks 'more painful' or 'sharper' than those in the forearm; two could detect no difference, and two thought the pricks were sharper in the forearm.

(3) *Cold.* The mean number of responses per 100 stimuli in the ear was 21·1; in the forearm it was 29·2. This difference was not, however, statistically significant. The mean threshold for cold in the ear was 27·8° C.; in the forearm it was 27·4° C.; these figures do not differ significantly.

Four out of ten subjects remarked a qualitative difference between the ear responses and the forearm ones, especially when the large stimulator tip was used; one of them expressed it by saying that the sensation in the ear was 'rather stinging cold, like a snowball', while the forearm sensation was not so definite. Three subjects considered the subjective judgements required were easier to make in the ear; two thought the forearm experiments easier and the remaining five had no preference.

(4) *Warm.* The mean number of responses per 100 stimuli in both the ear and the forearm was exactly the same, 8·9. The mean threshold for warm in the ear was 36·9° C., while in the forearm it was 35·2° C.; the difference was not of statistical significance. No subject could detect any qualitative difference between the two areas, but two thought the task of making a judgement was easier in the ear and one thought the reverse.

Comparison of auricle with finger

(1) *Touch.* In spite of the fact that a lighter thread was used, every stimulus applied to every ear was appreciated, whereas only 49 % of the stimuli on the finger were felt; the difference in favour of the ear was statistically significant ($p < 0{\cdot}01$).

(2) *Pinprick.* Four subjects considered the finger pricks were 'sharper' than those in the auricle; five could detect no difference and one thought the pricks were sharper in the auricle.

(3) *Cold.* The mean number of responses per 100 stimuli in the ear was 17·8; in the finger it was 21·1. This difference was not statistically significant. The mean threshold for cold in the auricle was 29·4° C.; in the finger it was 29·5° C.

Three out of ten subjects experienced a qualititative difference between auricle and finger, the ear sensations being more 'stinging'. The same three subjects also considered the subjective judgements were easier to make in the ear; the remaining seven had no preference.

(4) *Warm.* The mean number of responses per 100 stimuli was 8·1 in the ear and 6·0 in the finger; the mean threshold for warm in the ear was 38·3° C., while in the finger it was 39·5° C. In neither case is the difference of statistical significance.

Two subjects considered that in the ear the warm sensation rose more quickly to its maximum intensity than in the finger; the remaining eight could detect no qualitative difference. No subject had any preference in respect of the ease of making judgements.

Effect of chronological testing order

There was no evidence from the figures in any of the series of temperature experiments of any tendency towards either a 'learning' or a 'fatigue' effect in the testing area which was examined second.

B. *Histology*

(1) *Skin from the auricle*

The skin was smooth and thin, with many fine hairs. In the dermis, nerve fibres of various diameters were grouped into bundles of all sizes and formed a complicated cutaneous nerve plexus in every way similar to that in the dorsum of the ear of the rabbit (Weddell, 1941), except that, owing to the greater thickness of the human skin, it was more difficult to focus so many nerve bundles at a time.

Individual fibres terminated in one of two ways. Either they formed a complicated basket-like network around hair follicles (Fig. 2), or they ended freely after branching repeatedly. These free endings covered a wide area in the more superficial layers of the dermis and the deeper layers of the epidermis. The fibres from which they arose were either finely myelinated or non-myelinated, and were usually smooth in outline. In contrast, their successive branches, which were only clearly displayed in the methylene blue specimens (Weddell & Zander, 1950), were beaded (Fig. 3).

No 'organized endings'* were seen in any of the specimens examined. Occasionally something resembling one was noticed, but every such object could be shown to be an artefact, whose true nature could be made out by focusing up and down or by examining serial sections. In this way it was found that most of these artefacts were due to tearing of a fibre or fibre bundle in which the Schwann or surrounding connective-tissue elements were partially impregnated. Most of them occurred close to the plane of separation of the skin from the cartilage, and it is highly probable that their presence here was connected with the local damage inevitable at this point when the skin was stripped off (Weddell & Zander, 1950).

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* In this paper the term 'organized ending' refers to any closely knit and well-localized nerve termination (other than a simple terminal bead), whether or not it is surrounded by a connective-tissue capsule.

The regions surrounding the marked cold-sensitive spots did not appear to differ in any way from the rest of the specimen; there were certainly no 'organized endings' in relation to them.

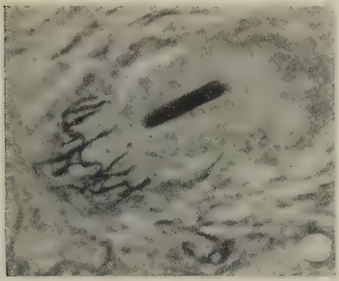

Fig. 2.

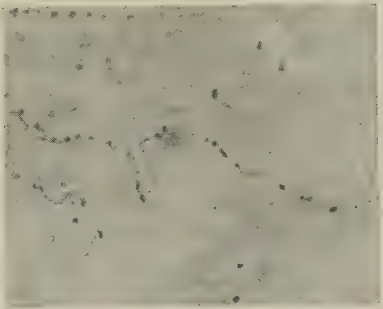

Fig. 3.

Fig. 2. Basket-like network of nerve fibres surrounding a hair follicle in the human auricle. Silver impregnation. (×350.)

Fig. 3. Free beaded nerve terminals in the epidermis of the human auricle. Methylene blue. (×650.)

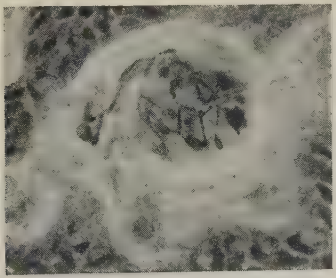

Fig. 4. Complex organized nerve ending from a dermal papilla in the human finger. Silver impregnation. (×650.)

(2) *Cartilage*

No nervous tissue of any kind was seen in the cartilage itself; there were nerve fibres accompanying the blood vessels in the perichondrium, but no organized endings.

(3) *Palmar aspect of the proximal phalanx of the ring finger*

The cutaneous plexus was qualitatively similar to that in the ear; the fibres, however, terminated differently. As in the ear, both myelinated and non-myelinated fibres gave rise to branches which terminated freely. The remaining fibres ended in the dermal papillae in a variety of organized endings (Fig. 4).

DISCUSSION

The human auricle is a region from which most of us have at one time or another experienced the sensations of touch, pain, cold and warm. In this investigation we have attempted to show that its sensitivity to these four classes of sensation is in no way unique, being of the same order as that of the skin on the front of the ring finger and the forearm. Indeed, the only statistically significant difference obtained was that the ear was more sensitive to touch stimuli of the type employed than the finger, and much more so than the forearm. It must be emphasized that no rigorous comparison was made, and in particular that there was no attempt to map temperature spots. The lability of such spots is well known (Dallenbach, 1927), and it would have been unnecessarily tedious to make a comparison on the basis of the number of spots in a given area. The point is not that the ear is more (or less) sensitive than the finger or forearm, but that tactile, painful and thermal stimuli can be appreciated when they are applied to the back of the auricle, and that, though there may be minor differences, there is apparently no gross qualitative peculiarity about the sensations so aroused.

The methods used to compare the sensory capacity of the ear with that of the control areas are only representative of many others which could be used; as psychological experiments they are open to many objections (the subjects were untrained; skin temperatures were not taken; the endurance and fatiguability of the subject were grossly involved; inadequate allowance was made for the relatively long reaction time to temperature; equivocal responses were dismissed; the pressure of the thermal stimulator was inconstant; the large size of the smaller temperature stimulus in relation to the grid permitted many grid squares to be stimulated at least twice: to name but a few). However, all these objections apply with equal force to the control area, and we may safely draw the conclusion that from the clinical and practical point of view touch, pain, warm and cold sensibility are adequately represented in the skin of the auricle.

The neural apparatus available in the auricle to mediate these sensations appears to comprise a series of complex basket formations round the numerous hair bulbs and a more widely dispersed network of fine undifferentiated terminals. No ending of any other kind was found, either in the skin on the side of the auricle tested, in the deeper tissues, or in the skin on the side remote from stimulation, in spite of the examination of over 30 sq.cm. of skin from twenty-one ears. The methods on which this result rests are those which were compared against phase-contrast appearances in the more favourable setting of the cornea (Weddell & Zander, 1950) and found to be mutually complementary. It cannot reasonably be argued that the silver impregnation failed to show up any 'organized endings', since numerous well-impregnated 'organized endings', both capsulated and non-capsulated, were found in the skin from the front of the finger.

We may therefore advance the main thesis of this paper, which is that, in the auricle at least, 'organized endings' are not essential to the perception of touch, pain, cold and warm stimuli. It is fairly certain that another such region exists in the vicinity of the inferior concha of the nose (Harpman, 1951), and such observations as those of Gilbert (1929) render it at least possible that the whole of the hairy skin constitutes a third. The auricle is, however, a particularly advantageous area in which to investigate the point, since the skin is thin and easily stripped from the underlying cartilage. There is little depth of dermis and subcutaneous tissue, and it is consequently easy to obtain and examine whole preparations including both skin and the entire underlying tissue.

Our findings thus serve to anchor to a more solid histological foundation the view current in psychological literature (Nafe, 1927, 1942; Kantor, 1947; Jenkins, 1951; Morgan, 1951) that 'organized endings' need not necessarily play any special part in sensory perception. This view has arisen out of repeated failure to relate any specific type of end organ to any specific type of sensation by the technique of biopsy of marked spots. This point is considered in more detail in another place (Weddell, Sinclair, Zander & Hagen, 1952), and it will suffice here to add our own failure to those of previous workers.

This is not, of course, to say that in those regions of the body where 'organized endings' do occur, such as the fingers, the lips, the conjunctiva, the genitalia, etc., they play no part in sensory discrimination. Nevertheless, we may conclude that nerve endings of a single morphological type are in certain regions concerned with more than one sensation (cf. Weddell, Sinclair & Feindel, 1948; Zander & Weddell, 1951). It may also be justifiable to infer that the morphology of a nerve terminal is not a factor of primary importance in determining its sensory specificity.

This conclusion runs directly contrary to the theory of cutaneous sensibility currently held by many physiologists and clinical neurologists and generally attributed to von Frey. The history of the development of this theory has been sketched by Boring (1942). It may perhaps be said to have sprung from the original clinical discovery that certain types or 'modalities' of cutaneous sensation could be separated from one another by the action of various diseases or physical agents. To this was added the doctrine of specific nerve energies attributed to Johannes Müller, the sensory investigations of such workers as Nothnagel (1867), and finally the discovery of the punctate representation of sensation in the skin by Blix (1884), Goldscheider (1884) and Donaldson (1885).

On these foundations the hypothesis was gradually erected that for each 'modality' of sensation a specific type of nerve-ending existed having morphological characteristics enabling its functional activity to be predicted. It was assumed that in relation to each punctate sensory 'spot' one or more end-organs of this type lay ready to initiate a train of events culminating in its possessor experiencing the specific sensation they subserved.

We do not at the moment wish to call in question either the validity or the usefulness of the clinical concepts of sensory 'modalities' or punctate sensibility, but merely to maintain, on a histological basis, that the nature of the neural apparatus in the human auricle negatives the universal application of the concept of morphologically specific receptors to the human skin. It might be possible to argue that the principle if not the details of the von Frey theory still hold, and that there are in fact different terminal mechanisms for the various modalities, but that in the auricle our microscopic technique is inadequate to distinguish them morphologically, though functionally they are distinct. This suggestion cannot be scouted, since the auricular innervation is indeed lavish and complex, and one cannot be certain that it is not composed of several elements. Nevertheless, it is an uncomfortable suggestion to be forced to make, and, since there are objections to the von Frey theory on other grounds (Ruffini, 1905; Nafe, 1927, 1929; Pradines, 1931; Kantor, 1947) it would appear safer to regard the whole structure of the theory—at least in so far as it involves specific peripheral receptors—as suspect.

SUMMARY AND CONCLUSIONS

The skin of the human auricle, when examined by the best available techniques for displaying peripheral neural elements, does not appear to contain any 'organized' nerve endings. There are only two types of peripheral termination: either the fibres end freely between the hairs or they supply a complicated basket-work round each hair bulb. Nevertheless, cutaneous sensibility in the auricle does not appear in any way peculiar, and touch, pain, cold and warm sensibility are adequately represented. A comparison shows that the sensitivity of the skin of the auricle to the four customarily recognized sensory modalities is comparable to that of the skin of the front of the forearm and the front of the ring finger. It therefore appears that the current theory of cutaneous sensation, which demands specific receptors for each modality of sensation, cannot be of universal application to the human body, and thus its standing must be seriously called in question.

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THE SHOULDER JOINT OF LIZARDS AND THE PRIMITIVE REPTILIAN SHOULDER MECHANISM

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INTRODUCTION

Recent interest in the mechanism of the limbs has developed partly from palaeontological and morphological studies, especially those of Watson (1917), Gregory & Camp (1918), Romer (1922), Miner (1925), and Schaeffer (1941), and also from mechanical analyses of the forces involved in tetrapodal progression, particularly by Manter (1938), Gray (1939, 1944) and Barclay (1946). The present contribution embodies a study of the lacertilian shoulder joint, in which the movements are sharply defined by strong, isolated ligaments, followed by an attempt to apply the results to the shoulder mechanisms of early tetrapods. The discussion necessarily involves a consideration of the gaits of the animals concerned.

THE SHOULDER OF TYPICAL LIZARDS

In *Varanus salvator*, a very large type with heavy bones and strong ligaments (Fig. 1), the head of the humerus consists of a single smooth articular condyle, narrow dorso-ventrally and elongated cranio-caudally, set off from the shaft by a neck directed dorsally and medially. The glenoid surface of the scapulo-coracoid looks laterally and caudally and is saddle-shaped, being concave dorso-ventrally, with prominent lips, and convex cranio-caudally. The joint surfaces are highly incongruent, and in most positions of the joint the humeral condyle can only rest on the lips of the saddle without touching the cartilage between. Without a knowledge of the arrangement of the ligaments, it would be difficult to judge the range of movements of such a joint.

There are in fact four ligaments, arranged as dorsal and ventral cruciate pairs, which may be named from their distal attachments as are the cruciate ligaments of the human knee. The cranio-dorsal ligament (Fig. 2, *cr.d.l.*) passes from the scapula, near its caudal margin, to a tubercle lying cranial to the articular condyle of the humerus, and crosses superficial to the shorter caudo-dorsal ligament (*cd.d.l.*), which passes from its attachment dorsal to the glenoid to an area caudal to the condyle. The cranio-ventral ligament (*cr.v.l.*) is long, passing from the caudal part of the coracoid plate to an attachment near the cranio-dorsal ligament, and crossing the shorter caudo-ventral ligament (*cd.v.l.*). These ligaments thus have a simple and symmetrical arrangement. A thin band joins the two posterior ligaments near their humeral attachments, and all four ligaments have twisted fibres.

In the mid-position of the joint, that in which the humerus lies in the middle of its range of movements, and from which it can be moved through an equal distance in all directions (Haines, 1944, p. 44), the humerus projects laterally and caudally from the body, and all four ligaments are relatively slack, so that, in the ligamentous preparation, the humerus can be pulled about 2 mm. away from the glenoid surface before they tighten. At the same time the two ventral ligaments and the dorsal lip of the glenoid saddle prevent the humerus leaving the glenoid dorsally, while the dorsal ligaments and ventral lip prevent dislocation ventrally. Cranio-caudal displacements are also controlled by the ligaments, the cranial pair preventing the humeral condyle from slipping cranially unduly, and the caudal pair preventing caudal dislocation, just as the cruciate ligaments of the mammalian ankle or human knee prevent similar displacements.

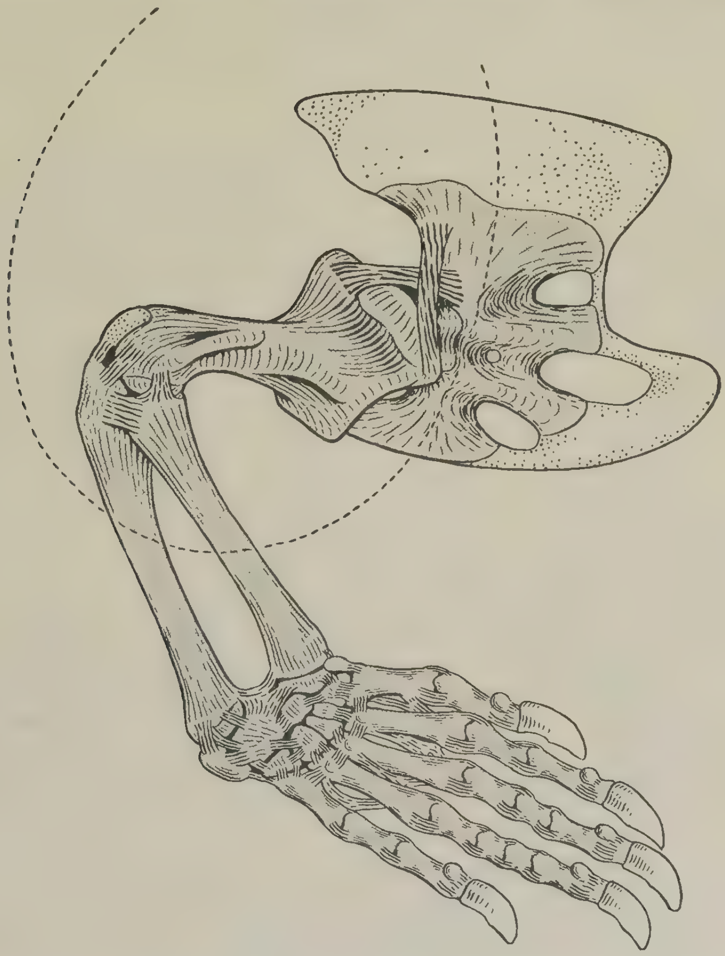

Fig. 1. Skeleton of the forelimb of the water monitor *Varanus salvator* in natural articulation.

Explanation of lettering for all figures:

Cd.	Caudal	*cr.d.l.*	cranio-dorsal ligament
cd.d.l.	caudo-dorsal ligament	*cr.v.l.*	cranio-ventral ligament
cd.v.l.	caudo-ventral ligament	*Ds.*	Dorsal
Cr.	Cranial	*Vn.*	Ventral

a–d in Figs. 3 and 4, *a–j* in Figs. 6 and 7, and *a*, *b*, *p*, *q* and *x* in Fig. 8, are explained in the text.

The distal end of the humerus describes a nearly circular course as it follows the limits of movement allowed by the ligaments (Fig. 1, broken line). The plane of the curve faces caudally rather than cranially, in accordance with the direction of the glenoid, and dorsally rather than ventrally, owing to the oblique setting of the head of the humerus on the shaft. The range of movement in both dorso-ventral and cranio-caudal directions is about one right-angle. As the humerus moves cranially (Fig. 2A), the caudal pair of ligaments is tightened and eventually limits the movement, but movement caudally is limited by contact of the limb with the body before the cranial ligaments become unduly tightened (Fig. 2B). Ventral and dorsal movements (Fig. 2C and D) are limited by the tightening of the dorsal and ventral pairs of ligaments respectively. The points of crossing of the ligaments alter with the varying positions of the joint.

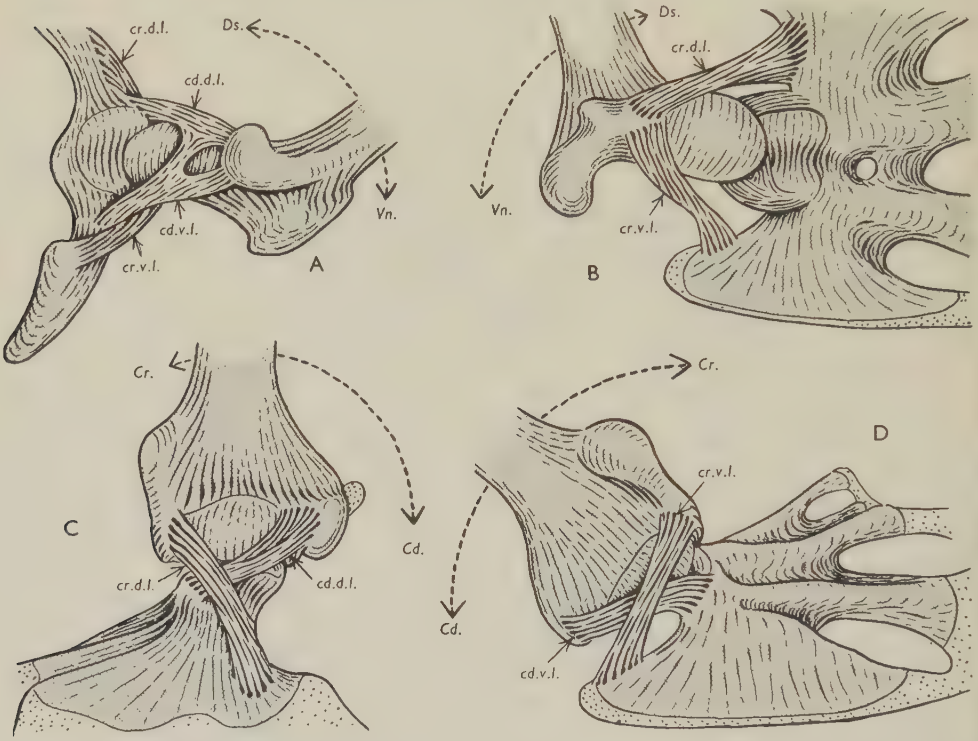

Fig. 2. Shoulder joint of *Varanus salvator*. A, caudal view with the humerus protracted, showing the caudal ligaments taut; B, cranial view with the humerus retracted; C, dorsal view; D, ventral view. The curved broken lines indicate ranges of movement.

In the mid-position of the joint, since all four ligaments are relatively slack, some rotation of the humerus (about 15°) about its own longitudinal axis is allowed. This is shown in Fig. 3 (position *c*) in the case of *Uromastix aegyptia* (= *spinipes*), a large burrowing herbivorous form, whose ligaments and movements resemble those of the *Varanus* already described. When the humerus of this animal is fully advanced (position *a*) the cranial margin of the head is firmly gripped by the glenoidal lips, and the caudal margin is held in position by the taut caudal ligaments, so that no rotation can occur, while in the intermediate position (*b*) rotation is very limited. But as the humerus swings caudally (to *d*) all the ligaments become slack, and the rotation then allowed increases to about 40°.

In both lizards the articular capsule is thin and membranous, and though it is attached to the posterior pair of ligaments, it can have no significant influence on the movements of the joint. In other lizards examined, including species of *Lacerta*, *Agama*, *Scincus* and *Iguana*, the ligamentous mechanism was similar, and, so far as can be judged from bony preparations illustrated by Camp (1923), El-Toubi (1949) and others, the lacertilian shoulder appears to be standardized in construction. Even in the thoroughly arboreal *Chamaeleo* the four ligaments can be recognized, though they are slacker than in typical lizards and allow the humerus to turn through an angle approaching a right angle (Fig. 4, *c* and *d*). The extra movement at the shoulder and the mobility of the wrist allow the hand to be placed so as to grasp

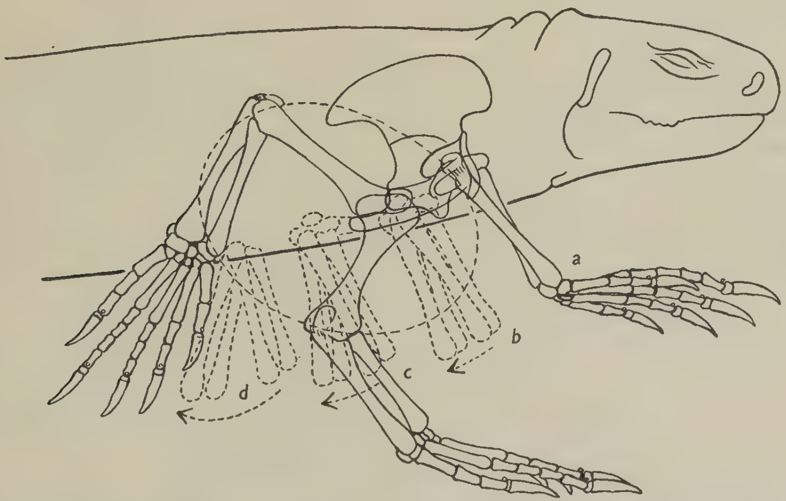

Fig. 3. Skeleton of the forelimb of the dab lizard, *Uromastix aegyptia*, showing the ranges of circumduction and rotation of the humerus.

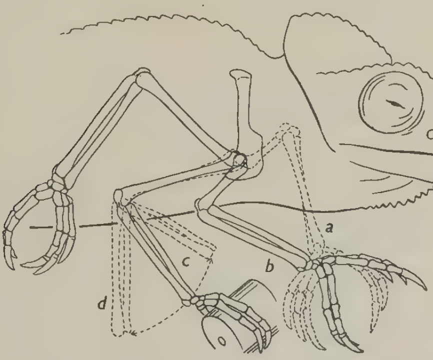

Fig. 4. Skeleton of the forelimb of *Chamaeleo vulgaris* showing the wide ranges of movement allowed at the shoulder.

objects placed in any direction in relation to the animal's trunk (*a* and *b*), which itself always remains upright.

The cruciate arrangement of the ligaments was not found in any of the Crocodilia or Chelonia examined. The Crocodilia are, however, believed to have passed through a bipedal, followed by a thoroughly aquatic, phase before adopting a secondarily bipedal gait (Huene, 1913), while the chelonian limbs are adapted to retraction within the carapace, so that neither group can be used to illustrate primitive conditions. The arrangement in *Sphenodon* is unknown.

THE GAIT OF MODERN REPTILES AND AMPHIBIANS

Huene (1913), from a detailed study of the crocodile, based particularly on the tracks in mud, found that the body was raised well away from the ground except for the tip of the tail. The feet, once they had been placed on the ground facing cranially, did not change their position, for the marks of the individual toes were not smudged. Reconstructions of early tetrapods showing the feet turning caudally during the stride were published by Gregory & Camp (1918, fig. 13) and Morton (1935, fig. 10), but Schaeffer (1941, fig. 7) gave drawings of the well-preserved tracks of *Ammobatrachus* showing that there was no turning even in early forms. From a study of simultaneous dorsal and lateral cinephotographs he was able to follow in some detail the course of a single step in *Alligator* (Schaeffer, fig. 17; present work, Fig. 5), and showed that the gait was very similar, stage for stage, to that of urodele amphibians, in which it had already been worked out, particularly as regards the synchronization of the movements, by Gray (1939). Schaeffer noted (p. 412) that 'the foot itself gradually rolls off the ground. . . . The foot remains in contact with the ground until the last phalanx of the longest digit has been turned plantar-side up, thus producing the greatest possible propulsive effort.' He also (p. 414) described the return of the limb to the forward position 'by a sort of crawl-stroke movement', with the digits loosely flexed on the palm during the greater part of the movement, but becoming extended before the foot touched the ground.

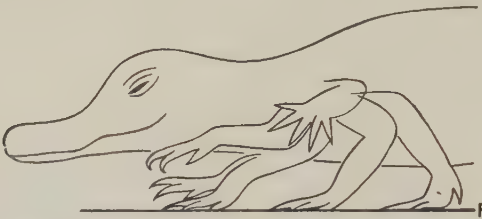

Fig. 5. Outlines of forelimb movements of a young *Alligator mississipiensis*, taken from Schaeffer's (1941) fig. 17, showing the hand 'peeling' off the ground and the 'crawl stroke' return movement.

No tracings from cinefilms have been published for lizards, where the movements are more difficult to photograph than in slower-moving animals. Visual observations made by the present author on captive animals, while walking or in arrested movement, showed that the gait was of the general type described by Schaeffer and the details he mentions were confirmed. Nothing is known, however, of the movements in running, but probably it is a simple development of the walking pace, as is the slow trot of mammals, there being nothing corresponding to the mammalian amble, rack or leaping-gallop (Gray, 1944; Slijper, 1946, p. 21).

Fig. 6 was prepared from a ligamentous preparation of *Varanus griseus*, a very active predator of the open desert, an animal which relies on its speed for escape from danger. The ligaments, not shown in the drawings, are similar to those of other lizards. The hand is placed on the ground with the digits spread, extended and facing cranially (*a*). The humerus is retracted along a roughly horizontal plane until it is directed laterally from the body, and at the same time the elbow is flexed so that the forearm passes ventrally and cranially, making an angle of about 45° with the ground (*b*). The humerus now moves caudally and somewhat dorsally, while the elbow continues to flex, though at a slower rate, until the wrist is at its nearest to the shoulder, and the two joints lie near the same vertical plane (*c*). The humerus now moves more dorsally than caudally, while the elbow begins to extend, so that the wrist moves away from the shoulder until the forearm becomes vertical, and at the same time the hand peels away from the ground (*d*). The humerus rises further, and extension of the elbow continues as the hand lifts off the ground, the shorter 1st and 5th digits losing contact before the others (*e*). Up to the moment of leaving the ground, the hand is moving caudally relatively to the trunk, as otherwise there would be a check to the smooth forward movement of the body.

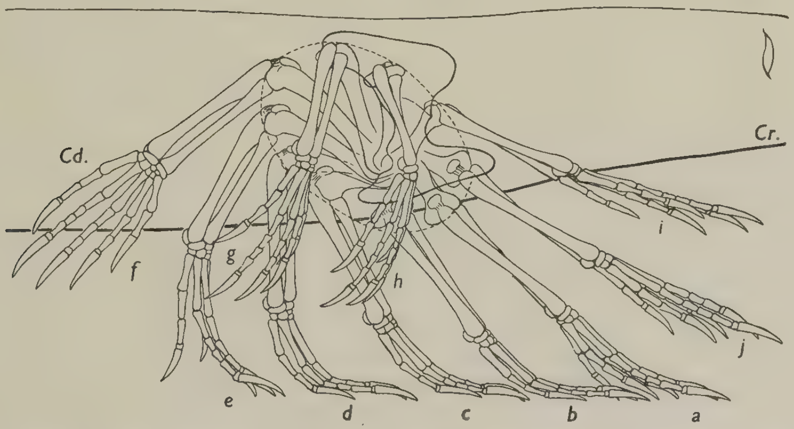

Fig. 6. Skeleton of the forelimb of *Varanus griseus*, showing the movements in a full pace.

After the hand has left the ground the humerus continues to rise, but at the same time turns cranially, while the elbow extends to a maximum and the palmar surface of the hand comes to face the trunk (*f*). A cranial movement of the humerus, with little or no alteration in the position of the elbow, carries the hand cranially, while the wrist and digits are loosely flexed, the wrist being at the same horizontal level as the shoulder, with the elbow well above (*g* and *h*). This corresponds precisely to Schaeffer's (1941) 'crawl-stroke' movement. The digits are extended and spread as the hand nears its most cranial position (*i*) and falls towards the ground (*j*). Contact with the ground is made first by the claws, but later the thick tissues covering the heads of the metacarpals come to take most of the weight. The hand is already travelling caudally as it makes contact, so that there is no check to movement. The drawing takes no account of lateral deviations of the trunk, which greatly increase the effective length of the stride in the living animal, nor of the variations in gait in the living animal, particularly in the height at which the limb is carried cranially (phases *g* to *i*).

The whole cycle can be imitated by a man facing a wall, for human joints allow all movements found in reptiles, besides others not possible to reptiles (Fig. 7). The proportions are of course different, the human hand in particular being relatively small, but this does not affect the demonstration, and the movements are easier to follow in the more familiar material.

As regards the lacertilian humerus there can be little doubt as to the sequence of cranio-caudal or dorso-ventral movements, but the rotations cannot be seen easily in the living animal. Study of the ligamentous preparation, however (Fig. 6), shows that from *a* to *c* there is little rotation, but from *c* to *e* a rapidly increasing rotation carries the forearm caudally on the distal end of the relatively slowly moving humerus. During the recovery phase, from *g* to *i*, this rotation is reversed to carry the forearm cranially near the head.

In *Bufo*, Barclay (1946, p. 198) compared the mechanism of the forelimb to that of a wheel having but one spoke, the rotating humerus representing the axle and the forearm the spoke, the tip of which was applied to the ground. In the shoulder joint, well known from the work of Braus (1910), who used *Bufo* for his experimental studies on the formation of joints, there are no cruciate ligaments, but the capsule is well developed. When the humerus is placed cranially a peculiar projection of the glenoid rim articulates with a groove in the head of the humerus so as to prevent rotation, but as the humerus is retracted the projection leaves the groove and the humerus is free to rotate (Barclay). Thus, although the joint construction is quite different in *Bufo* and *Varanus*, the movements of the two animals are very similar.

All authors are agreed that the body is pulled forward in the early part of the stroke by a flexion of the elbow (Fig. 6*a* to *c*). The pushing forward of the body towards the end of the stroke by an extension of the elbow (*c* to *e*) is less well known, but is shown clearly in Gray's (1939) photographs of *Triton* and Schaeffer's (1941) drawings of *Triturus* and *Alligator*. As regards movements within the forearm there are, however, great differences of opinion. Schaeffer (1941) believed that for the hand to have remained directed cranially throughout the period of contact with the ground, a high degree of pronation was required, particularly as the hand reached its most caudal position. His figure (3A) of a urodele, based on a study of cleared preparations, shows the radius at the end of the stroke crossing behind the ulna, a most peculiar position. Miner (1925) described no such extensive movements between the forearm bones in his detailed study of *Megalobatrachus*, and Haines (1946, p. 5) found that, in *Salamandra* and other urodeles, the movements could amount to only about half a right-angle, and (p. 9) that the only animals in which the forearm bones could turn about each other to the extent postulated by Schaeffer were the primates, where the mechanism was very highly specialized. In *Bufo*, where there can be no true pronation-supination, since the radius and ulna are fused together, the movement is replaced by a peculiar rotation of the combined bone on the lower end of the humerus (Barclay, 1946, p. 198, 'elbow rotation').

In lizards there is no indication of any considerable amount of radio-ulnar movement, nor would the joints allow it (Haines, 1946, p. 3). The apparent changes seen in the relative positions of the bones, such as those shown in Fig. 6 of the present paper, are due to shoulder and elbow movements that carry the forearm as a whole into its various positions relative to the observer. Further, a trial with the human model (Fig. 7) shows that there is little change in the relative position of the forearm bones as the hand is moved from *a* to *e*, for even at *a* the forearm is already nearly fully pronated and it remains so throughout the stroke. Circumduction and rotation of the humerus at the shoulder, combined with the requisite flexion or extension at the elbow, are sufficient to adapt the arm and forearm to the forwardly directed hand, without necessitating extensive movements of pronation or supination.

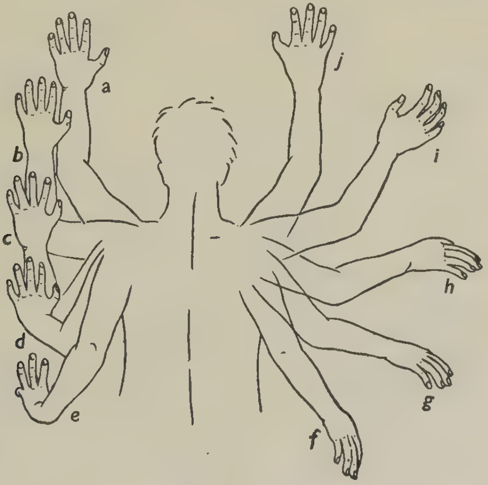

Fig. 7. **Human model of reptilian gait. The phases are labelled to correspond to those shown in Fig. 6.**

THE SHOULDER OF PRIMITIVE TETRAPODS

Watson (1917) first described in detail the peculiar screw-shaped glenoid and the twisted strap-like articular surface of the head of the humerus, found in early tetrapods, including both amphibians and reptiles. He considered that there was considerable cranio-caudal movement of the humerus at the shoulder with a coincident rotation, but believed (p. 4) that in such forms as *Eryops* and *Dimetrodon* the distal end of the humerus was 'confined to one definite track both in its forward and backward motion'. Romer (1922, p. 548) concluded, however, that walking demanded that, in addition to 'fore and aft motion', the humerus could be raised and lowered somewhat. He suggested that the glenoid was not a single screw-shaped surface, for that would entail great displacement of the surfaces and an undue amount of friction when the joint was moved. He believed rather that there were two separate surfaces, articulating with corresponding separate surfaces on the humerus. Miner (1925) made a very careful study of *Eryops*, in which, as Watson (1917) had already pointed out, ossification was especially good, and concluded that the glenoid surface was undivided, and that the screw-like and strap-like surfaces were concerned with imparting a rotation amounting to approximately a right-angle to the humerus as it was retracted. All these authorities considered the musculature, but not the ligaments, associated with the joint.

In view of the extraordinary conservatism found in other joints, particularly the knee, where the cruciate and collateral ligaments and the menisci have essentially the same arrangement in reptiles, birds and mammals, and were probably arranged in the same way in the early amphibians (Haines, 1942), it seems reasonable to suggest that in the shoulder the early reptiles may have had the cruciate arrangement found in modern lizards. At the hip a complex series of ligaments has been lost in passing from reptiles to mammals, apart from a remnant which helps to form the round ligament of the mammalian hip (Moser, 1893; Nauck, 1938). A full series of drawings of the pelycosaur *Dimetrodon* being available (Watson, 1917; Romer 1922), including details of the articular surfaces, a large-scale working model of the shoulder region was constructed in paper and paste, with the ligaments represented by cords placed as nearly as could be judged in the lacertilian position (Fig. 8). With two bands passing from the scapula, near its suture with the coracoid, representing the dorsal cruciate ligaments, and two from the coracoidal plate, one from near the supracoracoid foramen and the other from the caudal extremity of the coracoid, representing the ventral pair, the humerus was found to be held into the glenoid in all positions with no possibility of dislocation. Depression of the humerus tightened the dorsal pair of bands, and elevation tightened the lower pair, while cranio-caudal movements were checked by the posterior and anterior ligaments respectively. The cranio-caudal movements were carried out by rolling, rather than gliding, at the articular surfaces, and were controlled partly by the tensions of the cruciate ligaments and partly by the interlocking of certain small eminences which

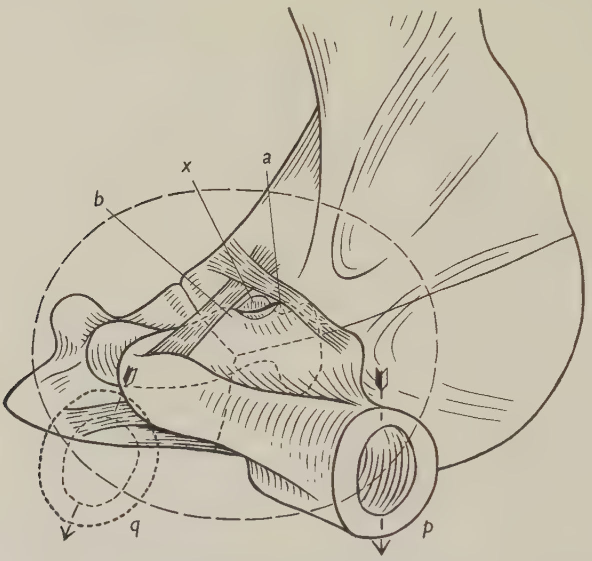

Fig. 8. Working reconstruction model of *Dimetrodon*, based on figures from Watson (1917) and Romer (1922).

are shown in detail by Romer (1922, figs. 2 and 3). A pair of these eminences (Fig. 8, present paper, *a* and *b*) on the dorsal surface of the humeral head interlocked with a projection (*x*) of the glenoid, and a similar pair on the ventral surface interlocked with another small projection of the glenoid. Thus there can be no question of excessive friction during this movement, and there is no need to postulate either excessive displacement or a double joint surface. Dorso-ventral movement did involve a gliding on each other of the articular surfaces.

The limits of movement followed a roughly circular track. Further, as the humerus moved caudally (from *p* to *q*), it rotated on its own axis, so that the ventral surface came to face caudally as well as ventrally, for the cranial end of the articular area was dragged downwards by the taut cranio-ventral ligament, while the caudal end was forced dorsally by the sloping caudal part of the screw-shaped glenoid.

Such a construction would give a workable joint suited to a gait similar to that seen in modern lizards and urodeles, and would agree well with the early tracks described by Schaeffer (1941). The humerus would move in a circular or oval course, rather than in a single line, and there would be no need to postulate any special movements of the forearm that are not found in modern types, such as the extensive pronation and supination suggested by Schaeffer, or the abduction and adduction at the elbow (forearm movements in a plane at right-angles to those of flexion and extension) suggested by Watson (1917, p. 13).

Whether the ligaments of early tetrapods did in fact resemble those of modern lizards must remain uncertain, nor can the precise ranges of the movements be determined, for slight changes in the lengths or positions of the ligaments may make great differences in the movements. But it seems unlikely that the mechanisms have changed in any fundamental way as between these two groups. In mammals, on the other hand, where a new type of gait has been evolved, in which the humerus swings more vertically beneath the glenoid, the whole set of ligaments has been lost.

SUMMARY

1. The shoulder joint of lizards is characterized by the presence of two pairs of cruciate ligaments, one ventral and one dorsal to the joint, whose positions and actions are described in detail.

2. The humerus can be circumducted in a roughly circular course, and can rotate on its own long axis.

3. As in other reptiles and in amphibians, the humerus is retracted and rotated during the propulsive phase of the stride, while the elbow is first flexed and then extended. In the recovery phase the limb is swept forward with the elbow but slightly flexed, while the rotation is reversed.

4. A model of the shoulder of the early tetrapod *Dimetrodon*, reconstructed with ligaments of the type found in lizards, allows cranio-caudal, dorso-ventral and rotatory movements suitable to a gait similar to that of modern reptiles and amphibians.

I wish to thank the officers of Abbassia Medical School and the Zoological Society of Egypt, and Prof. F. Davies and Dr E. Francis, of the University of Sheffield, for their gifts of material and help.

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VARIATIONS IN ORIGIN OF THE PARIETAL BRANCHES OF THE INTERNAL ILIAC ARTERY

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The first attempt to group the variations in the origin of the parietal branches of the internal iliac artery into definite patterns was undertaken by Jastschinski (1891 *a*). In an extensive study of the internal iliac artery in Polish subjects he classified the vessels into three categories:

(1) Those of large calibre (superior gluteal, inferior gluteal and internal pudendal arteries).

(2) Those of medium calibre (obturator artery).

(3) Those of small calibre (iliolumbar and lateral sacral arteries).

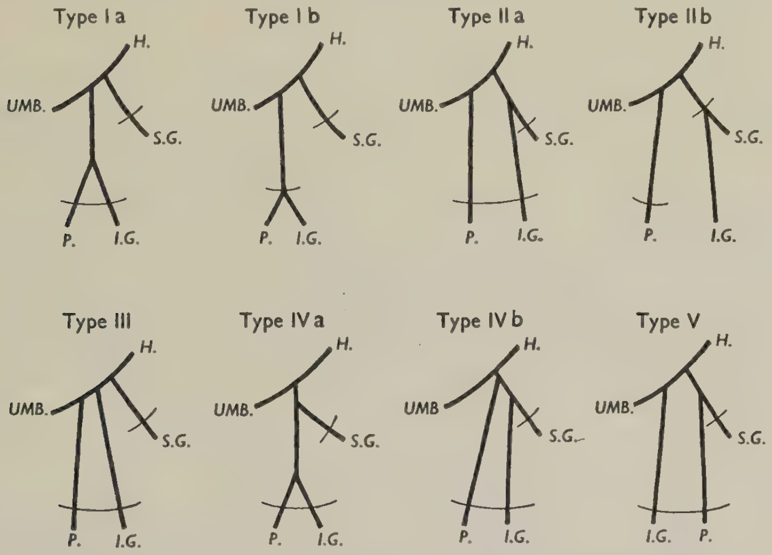

Fig. 1. Adachi's types. *H.* internal iliac artery; *I.G.* inferior gluteal artery; *P.* internal pudendal artery; *S.G.* superior gluteal artery; *UMB.* umbilical artery.

Jastschinski (1891 *a*) found that only the vessels in the first category showed sufficient regularity in origin to enable them to be grouped into definite *Types*, of which he described four. Adachi (1928) modified the method slightly, adding a fifth type of variation and included certain subtypes, in a study of the internal iliac artery and its branches in Japanese subjects. His scheme is as follows (Fig. 1):

Type I. The superior gluteal artery arises separately from the internal iliac artery, and the inferior gluteal and internal pudendal vessels are given off by a common trunk. If the latter divides within the pelvis it is considered to be *Type I a*, whereas if the bifurcation occurs below the pelvic floor it is classified as *Type I b*.

Type II. The superior and inferior gluteal arteries arise by a common trunk and the internal pudendal vessel separately. In this category, as in the previous one, two subtypes are described. *Type IIa* includes those specimens in which the trunk common to the two gluteal arteries divides within the pelvis, and *Type IIb* those in which the division occurs outside the pelvis.

Type III. The three branches arise separately from the internal iliac artery.

Type IV. The three arteries arise by a common trunk. The subtyping in this group is based on the sites of origin of the superior gluteal and the internal pudendal arteries from the parent stem. In *Type IVa* the trunk first gives rise to the superior gluteal artery before bifurcating into the other two branches; in *Type IVb* the internal pudendal is the first vessel to spring from the common trunk, which then divides into superior and inferior gluteal arteries.

Type V. The internal pudendal and the superior gluteal arteries arise from a common trunk, and the inferior gluteal has a separate origin.

Ashley & Anson (1941), who carried out a similar investigation to that of Lipshutz (1918) in American subjects (Whites and Negroes), employed the umbilical artery in addition to the three large parietal trunks for 'typing' and the obturator artery for 'subtyping' the internal iliac variations.

Since the variations in origin of the parietal branches of the internal iliac artery are of great surgical importance, but have not been previously investigated in a large series of British subjects, it was decided to undertake this extensive study.

On account of the marked variations in origin of the obturator artery the findings for this vessel will be described separately from those pertaining to the superior gluteal, inferior gluteal and internal pudendal arteries, and the latter will be classified according to Adachi's method. The existing arterial variations can be as well appreciated by this approach as by employing the obturator artery as an additional factor in 'subtyping', as in the classifications of Lipshutz (1918) and Ashley & Anson (1941).

MATERIAL AND METHODS

A total of 169 pelvic halves have been studied, of which seventy-four pelves were examined completely (i.e. 148 pelvic halves), the remaining twenty-one being single sides. Of these sixty-one were female (twenty-seven adult and thirty-four infant pelvic halves), and 108 (ninety-one adult and seventeen infant) were male.

The adult material consisted of subjects from the post-mortem and dissecting rooms, between the ages of thirty-two and eighty-nine. The post-mortem room pelves were injected with a carmine gelatine preparation through one internal iliac artery, to display more clearly the pelvic arteries, indian ink ('Raven' brand) being employed for the injection of all the infant material.

Photographs or drawings were made after displaying the branches of the internal iliac artery. Their sites of origin from the parent trunk, their destinations and any abnormalities found were carefully recorded.

FINDINGS

(1) *Superior gluteal, inferior gluteal and internal pudendal arteries*

The findings in 169 observations are given in Table 1, and examples of some of the variations noted in the present series are depicted in Figs. 2–5.

Table 1. *Internal iliac types*

	Female		Male		Total	
Types	No.	%	No.	%	No.	%
I *a*	25	40·9	57	52·7	82	48·5
I *b*	5	8·2	12	11·1	17	10·0
II *a*	11	18·0	9	8·3	20	11·8
II *b*	4	6·5	2	1·8	6	3·5
III	13	21·3	25	23·1	38	22·5
IV *a*	2	3·2	2	1·8	4	2·4
IV *b*	1	1·6	1	0·9	2	1·2
V	—	—	—	—	—	—
Totals	61	99·7	108	99·7	169	99·9

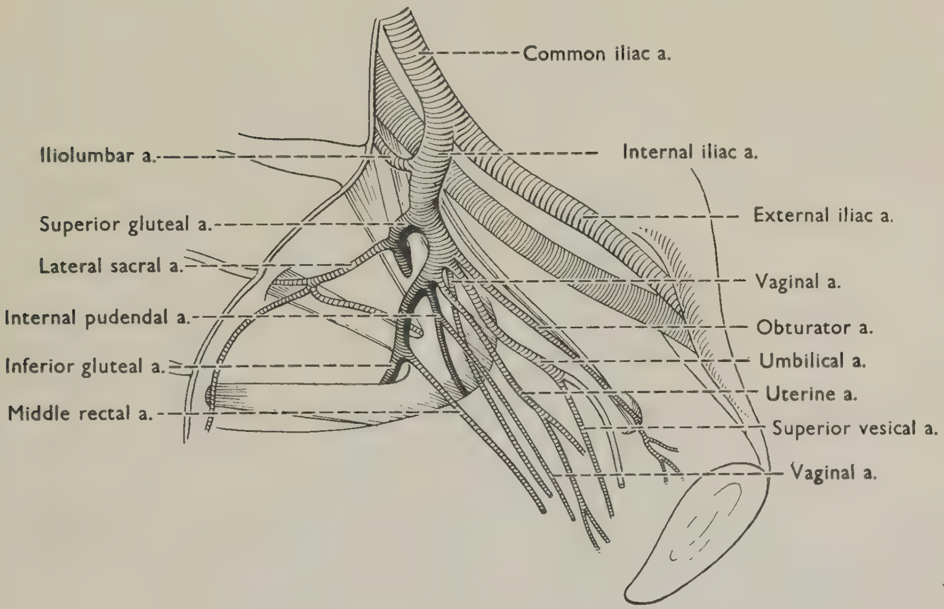

Fig. 2. Internal liac artery—*Type I a.*

A Type I arrangement is the most frequent finding, accounting for 58·5 % of all specimens; the common stem for the inferior gluteal and internal pudendal arteries divides proximal to the pelvic floor in 48·5 % of instances, whilst in 10 % the division occurs outside the pelvis. Type III is found in 22·5 % and a Type II arrangement in 15·3 %; in the latter the trunk common to the two gluteal arteries divides proximal to the pelvic floor in 11·8 % of instances and below this level in 3·5 %. The pattern conforming to Type IV is comparatively rare, being present in only 3·6 % of specimens. A Type V arrangement is not found in this series.

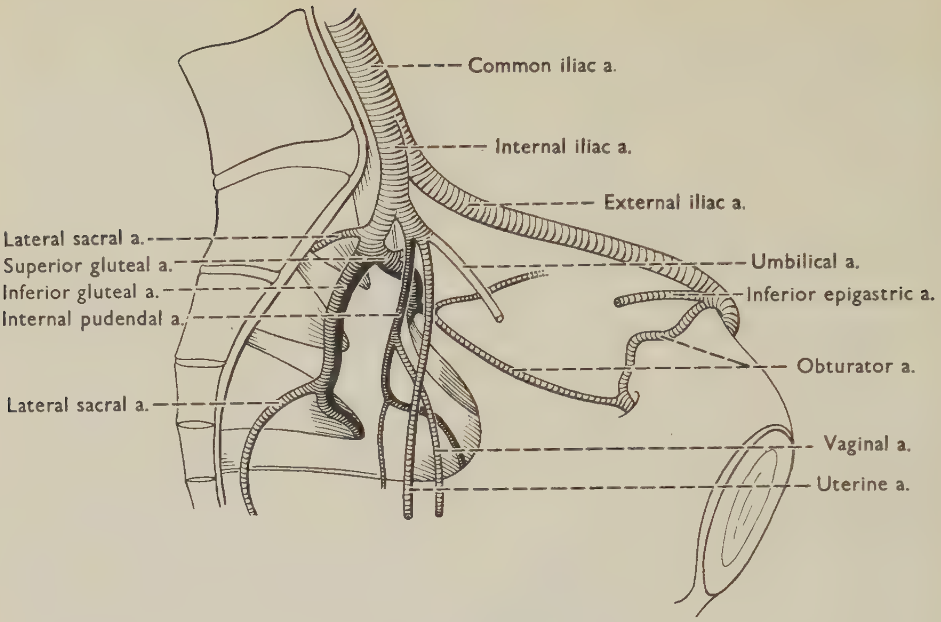

Fig. 3. Internal iliac artery—*Type IIa.*

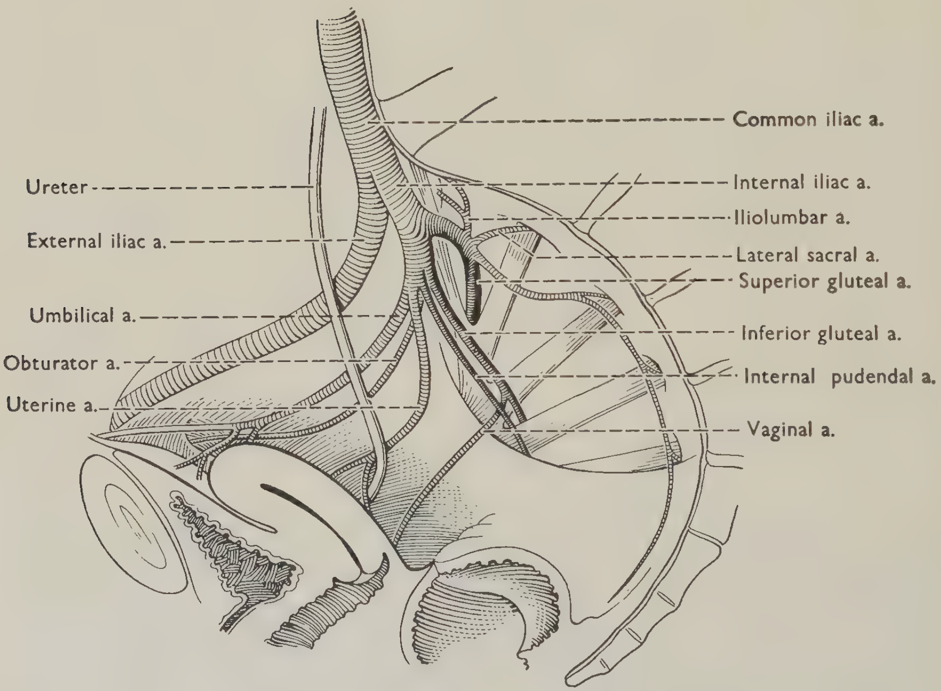

Fig. 4. Internal iliac artery—*Type III.*

The main difference between the female and male series is in the proportion of Type II arteries, only 10·1 % of the male specimens being in this category as compared with 24·5 % of female.

Thirty-nine of the seventy-four complete pelves examined (52·7 %) exhibit a similar origin of the three parietal arteries on both sides (twenty-seven out of forty-nine male pelves (55 %) and twelve out of twenty-five female pelves (48 %)).

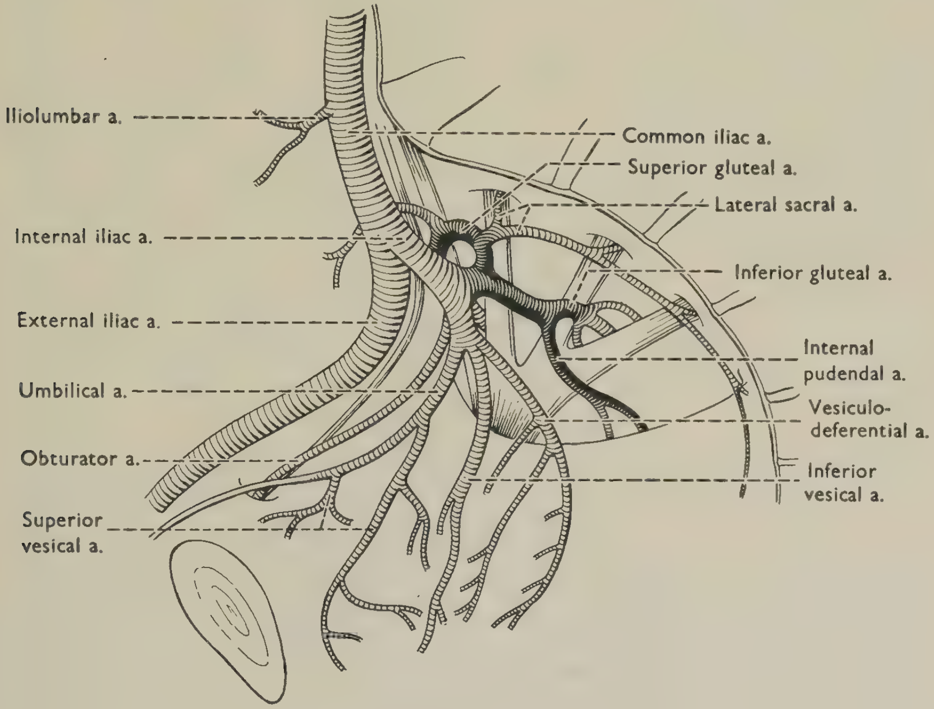

Fig. 5. Internal iliac artery—*Type IVa.*

(2) *Obturator artery*

The findings in 169 observations are given in Table 2, and represented diagrammatically in order of frequency in Fig. 6.

The obturator artery is most frequently a direct branch of the anterior division of the internal iliac artery, in 41·4 % of instances, and arises from the inferior epigastric artery in 19·5 %. There is a similarity of incidence of origin (10 % of cases) of the obturator artery from the superior gluteal and inferior gluteal-internal pudendal arteries. In 6·5 % of instances the obturator artery arises by a bifid root, one from an internal iliac source and the other from the external iliac artery. Less frequently, it is given off by the inferior gluteal artery (4·7 %), the internal pudendal artery (3·8 %), and as a direct branch from the external iliac artery in 1·1 %. The main difference between the two sexes is in the incidence of a superior gluteal origin; this occurs in 16·4 % of the female specimens as compared with 6·4 % of male. Of the seventy-four complete pelves examined, the obturator artery exhibits a similar origin on both sides in only 23 %.

Table 2. *Origins of obturator artery*

Origin	Female No.	Female %	Male No.	Male %	Total No.	Total %
A. From internal iliac artery:						
(i) Direct branch from anterior division	24	39·3	46	42·6	70	41·4
(ii) Inferior gluteal-internal pudendal trunk	5	8·2	12	11·1	17	10·0
(iii) Inferior gluteal artery	2	3·2	6	5·5	8	4·7
(iv) Internal pudendal artery	2	3·2	4	3·7	6	3·8
(v) Superior gluteal artery	10	16·4	7	6·4	17	10·0
(vi) Iliolumbar artery	3	4·9	2	1·8	5	3·5
B. From external iliac artery:						
(i) Direct branch	1	1·6	1	0·9	2	1·1
(ii) Inferior epigastric artery	10	16·4	23	21·3	33	19·5
C. From internal and external iliac arteries:						
By double origin	4	6·4	7	6·4	11	6·5
Totals	61	99·6	108	99·7	169	99·5

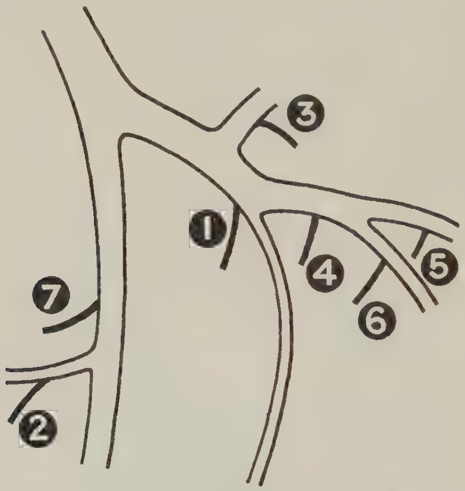

Fig. 6. *Origins of the obturator artery* (in order of frequency). 1, from anterior division of internal iliac artery—41·4%; 2, from inferior epigastric artery—19·5%; 3, from superior gluteal artery—10%; 4, from inferior gluteal-internal pudendal trunk—10%; 5, from inferior gluteal artery—4·7%; 6, from internal pudendal artery—3·8%; 7, from external iliac artery—1·1%.

DISCUSSION

Although the mode of origin of the three large parietal vessels conforms to one of four types in the present investigation, an arrangement in which the superior gluteal artery arises proximal to the common trunk for the inferior gluteal and internal pudendal arteries is comparatively constant (58·5%). This finding is in agreement with Adachi (1928) and Ashley & Anson (1941). Jastschinski (1891*a*), however, notes a Type I incidence in only 38% of his specimens. The obturator artery is much more variable. All investigators are agreed that the most common site of origin of this vessel is from the anterior division of the internal iliac artery as a direct branch; present findings, however, differ from those of previous observers in the comparatively low incidence of an obturator artery arising from the inferior epigastric artery (19·5 %), and the high incidence of a vessel with a double origin (6·5 %).

The incidence of an origin from the inferior epigastric artery varies from 20 to 30 % in most series (Jastschinski, 1891*b*; Quain, 1844; Levi, 1901; Pick, Anson & Ashley, 1942). Most observers describe the obturator vessel as having a double origin in about 1 % of cases (Quain, 1844; Dwight, 1895; Pick *et al.* 1942). Adachi (1928) pointed out that an obturator artery with two roots probably occurs more frequently than previous findings have indicated, this discrepancy being due to the difficulty of recognizing one of the roots when of very small size. In such instances, the larger root, which generally arises from the inferior epigastric artery, would probably be regarded as an obturator artery arising by a single stem.

In the present investigation, many of the pelvic arteries were injected in fresh material prior to dissection; this enabled the fine vessels to be followed more accurately than is possible in uninjected dissecting room material, from which earlier investigators have obtained their results.

It is suggested, therefore, that the technique adopted may account for the recognition of a greater number of obturator arteries with a double origin compared with other findings and, consequently, a lower incidence of single obturator vessels of inferior epigastric origin.

SUMMARY

1. The sites of origin of the superior gluteal, inferior gluteal, internal pudendal and obturator arteries have been investigated in 169 specimens (108 male and sixty-one female).

2. The superior gluteal, inferior gluteal and internal pudendal arteries are comparatively constant in their origins, conforming to a Type I arrangement on the Adachi scale in 58·5 % of cases, a Type III pattern being found in 22·5 % and Type II in 15·3 %. Type IV is less frequent and occurs in 3·6 % of specimens. In 52·7 % of instances, a similar origin of vessels is noted on both sides.

3. The obturator artery is more variable and arises as a direct branch from the anterior division of the internal iliac artery in 41·4 % of instances, from the inferior epigastric artery in 19·5 %, from the superior gluteal artery in 10 %, from the inferior gluteal-internal pudendal trunk in 10 % and by a double origin in 6·4 %. In only 23 % of instances is a similar origin noted on both sides.

4. The present findings show a lower incidence from the inferior epigastric artery and a higher rate of occurrence of a vessel with a double origin than previous series. Reasons are suggested to explain these differences.

I wish to thank Prof. R. G. Harrison for his helpful criticism of this work, also Prof. G. A. G. Mitchell of Manchester University, in whose Department the greater part of this study was carried out, for allowing me all facilities.

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DEVELOPMENTAL CHANGES IN THE OESOPHAGEAL EPITHELIUM IN MAN

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INTRODUCTION

The development of the oesophageal epithelium has been discussed by Schaffer (1904), Schridde (1904, 1907), Johnson (1910), and Lewis (1910), that of the superficial (or cardiac oesophageal) glands by Schaffer (1904) and Boerner-Patzelt (1922), and that of the deep or submucous oesophageal glands by Schaffer (1904) and Johnson (1910).

The origin of the upper group of the superficial glands is disputed. Schaffer (1904) maintained that they arose in areas of single-layered, tall columnar epithelium which appear in the upper end of the oesophagus in the fourth month. Such an epithelium in this situation was described by d'Hardivillier (1897) in a seventh-month embryo, by Patzelt (1921) in a third-month embryo and by Boerner-Patzelt (1922) in a series of embryos ranging from 78 mm. to full term. On the other hand, Schridde (1904) considered that the superficial glands arose from the basal layer of the endoderm, such a developmental pathway being allowed by the failure of the downward migration of stratified squamous epithelium from the oral cavity to invade certain 'protected' areas in the lateral angles of the oesophagus. However, Patzelt (1921) has disputed that such a migration of stratified squamous epithelium from the oral cavity to form the oesophageal lining occurs.

The occurrence of ciliated epithelium in the embryonic oesophagus was described by Schaffer (1904), Neumann (1876), Jahrmaeker (1906) and Johnson (1910). Eberth (1897) considered that the superficial oesophageal glands could arise from such epithelium, although Neumann (1897) disputed this and considered that the ciliated epithelium could give rise to the stratified squamous epithelium by loss of cilia and subsequent flattening of the cells. Johnson (1910) maintained that the ciliated cells and the stratified squamous cells arose simultaneously, growth of the latter then crowding out the ciliated cells.

The purpose of the present work was to examine the developmental changes that occur in the oesophageal epithelium, with particular reference to the development of the superficial glands.

MATERIAL AND METHODS

Serial transverse sections of the oesophagus in human embryos of the following stages, expressed as C.R. lengths (mm.), were examined: 3, 5, 5·5, 7, 9, 11·5, 13, 16 (two specimens), 23, 25, 34, 40, 42, 62, 72, 78, 90, 110, 130, 160, 162, 172, 210 and 230. In addition, serial transverse sections of the oesophagi of four full-term foetuses were examined. Up to the 42 mm. stage, whole embryos were serially sectioned at 10 μ, while in those of greater length the oesophagus was removed by dissection, complete with its attachments to the pharynx, larynx and trachea above, and to the cardiac end of the stomach below, and then serially sectioned at 10 μ.

Serial transverse sections of the oesophagi of four subjects aged 2, 11, 47 and 58 years respectively, and every tenth section of a transverse series from the upper and the lower thirds of the oesophagi of twenty subjects, ranging from 10 to 60 years of age, were also examined.

In general, the sections were stained with haematoxylin and eosin. Some were treated with the periodic acid—Schiff method (McManus, 1946), or by the Gomori method (Gomori, 1946) for the demonstration of mucin.

OBSERVATIONS

At the 3 mm. stage the epithelium lining the oesophagus is stratified columnar in type, being in general three cells deep, and oval nuclei are scattered irregularly through the entire thickness of the epithelium (Pl. 1, fig. 1). At the 5 mm. stage the epithelium has increased in thickness, though it is still only three cells deep, and is uniformly thick over the whole length of the oesophagus. Coincident with the increase in thickness there is a narrowing of the lumen which occurs to the greatest extent at the level of the tracheal bifurcation, this being the condition in all the embryos between the 5 and 23 mm. stages. In these embryos also, the diameter of the lumen above the level of the tracheal bifurcation is greater than that below. In the 7 mm. embryo (Pl. 1, fig. 2) the epithelium has a basal layer of columnar cells, and this is also present in the 9 mm. and the 11·5 mm. embryos (Pl. 1, fig. 3); the epithelium is still, in general, three cells deep.

In the 13 mm. embryo there is a cavity between the epithelial cells immediately above the level of the tracheal bifurcation (Pl. 1, fig. 4). This is the first stage in the process of vacuolation observed in the present specimens. The cavity is confined to four sections in its vertical extent, but as seen in transverse section it is nearly as big as the oesophageal lumen itself. The epithelium has the same appearances in this and the 16 mm. embryos as in the younger specimens. In the 16 mm. embryos the vacuoles are larger, are present in all parts of the oesophagus, and in places produce irregularities in the outline of the oesophageal lumen.

In the 23 mm. embryo (Pl. 1, fig. 5) there is a distinct change in the appearance of the epithelium, which is now in the form of two layers of columnar cells; the nuclei have also changed from an oval shape to become circular and smaller. Vacuolation is well established throughout the entire length of the oesophagus; above the level of the tracheal bifurcation the vacuoles occupy almost exclusively the lateral walls, but at and below this level they are present on all the walls, those on the ventral wall being smaller than those elsewhere. The vacuoles attain their largest size in the 25 mm. embryo (Pl. 1, fig. 6), in which they are also more numerous, and, particularly in the lateral angles of the upper part of the oesophagus, some possess a diameter greater than that of the oesophageal lumen itself. The oesophageal epithelium on the deep aspects of the vacuoles is thinner than elsewhere, and the nuclei show crowding in this situation. The interiors of the vacuoles are separated from the oesophageal lumen by epithelial partitions which are thinnest in relation to the largest vacuoles. After this stage, the vacuoles are smaller and less numerous, but minute vacuoles are present in the upper part of the oesophagus until the 72 mm. stage (Pl. 2, figs. 10, 12), subsequent to which they were no longer observed.

In the 34, 40 and 42 mm. embryos the epithelium is stratified columnar, and is thicker than in the 25 mm. embryo, being in general four cells deep. In the upper part of the oesophagus in these three embryos, the epithelium varies in thickness, areas of a two-layered columnar epithelium lying side by side with thicker areas possessing many layers. In the 40 mm. embryo, ciliated cells form the superficial stratum of the stratified epithelium over discrete areas on the dorsal and ventral walls of the middle third of the oesophagus (Pl. 1, fig. 7). In the 42 mm. embryo, the entire middle third of the oesophagus is lined on all its walls by stratified columnar ciliated epithelium; above and below this region there are patches of ciliated cells in an otherwise non-ciliated epithelium. The ciliated cells stand out sharply from those subjacent to them by their deep cytoplasmic staining with eosin. In places the surface continuity of the ciliated cells is interrupted by clear cells with sharp cell outlines and pyknotic nuclei; they project into the lumen of the gut beyond the level of the surrounding epithelium (Pl. 1, fig. 8). In the 62 mm. embryo the entire length of the oesophagus is lined with stratified columnar ciliated epithelium, but at its upper and lower ends there are areas (Pl. 2, fig. 9) in which the epithelium is composed of a single layer of tall columnar cells. At the upper end of the oesophagus these areas occupy the lateral angles and the adjacent parts of the dorsal and ventral walls, while at the lower end they lie between the longitudinal folds of the mucous membrane.

In the 72 and 78 mm. embryos simple columnar epithelium is present in the same areas as in the 62 mm. stage (Pl. 2, fig. 10). The cells have increased in height and their nuclei now occupy a basal position in the cells. They vary in height, being taller in the centres of the areas and shorter towards the periphery. In the 72 mm. embryo the margins of the areas of columnar epithelium are separated from the ciliated epithelium, which lines the rest of the oesophagus, by a narrow zone of non-ciliated stratified columnar epithelium (Pl. 2, fig. 12). This zone is absent in the 78 mm. embryo and the margins of the areas of columnar epithelium are directly bordered by ciliated epithelium. Although the simple columnar epithelium in the upper end of the oesophagus in the 72 mm. embryo is present in both lateral walls, it has a greater oral and caudal extension on the right side. At the lower end of the oesophagus it is arranged in the form of longitudinal columns between the folds of the mucous membrane, and is continuous with the simple columnar epithelium of the stomach, with no line of demarcation between the two. All the cells of the simple columnar epithelium contain mucin (Pl. 2, fig. 11). In the 90 mm. embryo these columnar cells are even taller than in the 70 mm. stage, so that they project above the general level of the surrounding ciliated epithelium (Pl. 2, fig. 13). The total area occupied by the columnar cells is smaller than in the 72 mm. embryo, and close to the margins of the main columnar cell areas there are small patches of simple columnar epithelium in which the cells themselves are shorter, the cell boundaries less clearly defined and the cytoplasm stains pale blue with haematoxylin, in contrast with the clear and unstained appearance of the tall cells in the main masses (Pl. 3, fig. 19). These smaller areas vary in size, but their average dimensions are

80 × 60 μ. Elsewhere the oesophagus is lined with stratified columnar ciliated epithelium. All the cells of the simple columnar epithelium, irrespective of their microscopic appearance and staining characteristics, give a positive reaction for the presence of mucin with the tests employed. The 110 mm. embryo shows similar appearances except that the areas of simple tall columnar epithelium are not so extensive, and many groups of shorter columnar cells, like those described above, are found close to the margins of the main areas. In this embryo one well-differentiated mucous gland was found lying deep to the developing muscularis mucosae, immediately below the level of the cricoid cartilage.

In the 130 mm. embryo the vertical extent of the areas of tall columnar epithelium at the upper and lower ends of the oesophagus are further reduced. At the lower end, the longitudinal columns of this epithelium have in part lost their continuity with the epithelium of the stomach, so that small discrete patches remain, and extend for about 1 mm. above the cardio-oesophageal junction. In the right lateral angle of the oesophagus, opposite the level of the first tracheal cartilage, there is a tubular downgrowth of tall columnar cells (Pl. 2, fig. 14). In the present series this is the first stage in the development of the superficial glands. The downgrowth extends to, but does not pierce, the developing muscularis mucosae, and its orifice is surrounded by a narrow zone of tall columnar cells. In the upper and lower thirds of the oesophagus, areas of shorter columnar cells, which show indistinctness of cell outlines and blue-staining cytoplasm, are frequent, and in places these cells project into the lumen of the oesophagus, as if pushed lumenwards by the subjacent cells (Pl. 3, fig. 20). In the same regions of the oesophagus are found minute circular cavities, lined with ciliated cells, lying within the thickness of the stratified columnar ciliated epithelium which forms the major part of the lining. The cavities are more numerous in the upper part of the oesophagus (Pl. 4, fig. 21). In the middle third the epithelium is stratified squamous in type and occurs in discrete patches, separated by thinner areas of stratified columnar ciliated epithelium.

In the 160 mm. embryo the middle third of the oesophagus is lined solely by stratified squamous epithelium. At the upper and lower ends are areas of simple tall columnar epithelium which are of lesser extent than in the previous embryo. Elsewhere the epithelium is stratified columnar ciliated. In this embryo a superficial gland, possessing five acini, was found in the right lateral angle of the upper oesophagus at the level of the third tracheal cartilage (Pl. 3, fig. 15); similar glands were found at the lower end of the oesophagus. The tall columnar cells composing the glands give a positive reaction for the presence of mucin. In the 162 mm. embryo the tall columnar epithelium is rather more extensive, being 0·71 mm. in vertical extent at the upper end, and 0·65 mm. at the lower end of the oesophagus. No oesophageal glands were found in this embryo. Again, in the 172 mm. embryo, the tall columnar epithelium is extensive, being comparable in extent with that described in the 72 mm. embryo and having a vertical extent of 1·0 and 1·25 mm. in the left and right lateral angles respectively of the upper oesophagus, and 1·20 mm. at its lower end. Isolated groups of shorter columnar cells, close to the margins of the main tall columnar areas, are frequent. In these main areas, at the level of the second tracheal cartilage, there are superficial glands which project against, and are partially enclosed by, the developing muscularis mucosae, but which do not pierce it to reach the submucosa (Pl. 3, figs. 16, 17). Similar glands are found at the lower end of the oesophagus. In the 172 mm. embryo stratified squamous epithelium lines the middle third of the oesophagus, and stratified columnar ciliated epithelium persists at either end, though more extensively above.

In the 210 mm. embryo there are numerous superficial glands on the right side extending from the level of the cricoid cartilage to that of the second tracheal cartilage. They open on to a surface composed of tall columnar cells (Pl. 3, fig. 18). On the left side, superficial glands are also present between the levels of the third and fourth tracheal cartilages; they open on to a surface which is lined by either stratified columnar ciliated or stratified squamous epithelium. Similar glands are also present at the lower end of the oesophagus, which has now lost its ciliated lining and is lined by stratified squamous epithelium. In the upper end of the oesophagus the epithelium varies greatly in thickness in adjacent areas, thicker areas of stratified squamous epithelium being intermingled with thinner areas of stratified columnar ciliated epithelium.

The oesophagus of the 230 mm. embryo has a general lining of stratified squamous epithelium, amongst which relatively thin areas of stratified columnar ciliated epithelium persist in the upper part only. At the level of the first tracheal cartilage on the left side there is a small superficial gland, consisting only of a single acinus. Otherwise no trace of tall or short simple columnar epithelium persists in the upper part of the oesophagus. At the lower end of the oesophagus, superficial glands are present, though few in number, their orifices being surrounded by either stratified squamous or simple columnar epithelium.

No deep glands were found in any of the embryonic material examined, other than that described in the 110 mm. embryo.

The four full-term specimens have superficial glands at the lower end of the oesophagus, all limited to a zone within 0·5 cm. of the cardio-oesophageal junction. Three of the specimens have superficial glands at the upper end of the oesophagus, but they are not prominent and consist of a few acini only. Two of the specimens have deep glands. In one of these, a single tubular downgrowth (Pl. 4, fig. 24) from the stratified squamous epithelium lies in the middle of the oesophagus. At its distal end it divides into three finger-like processes which show no differentiation into mucous cells; the cells gave a negative reaction for the presence of mucin, in contrast to the positive reaction given by the deep glands of the adult. In the second specimen, one gland of this type was found in the middle of the oesophagus, and another, showing differentiation into mucous cells, was noted immediately below the level of the cricoid cartilage.

The general histological characteristics of the superficial and deep glands in the adult are well known and need not be described. The postnatal material in the present series shows that the superficial glands are most commonly represented by minute glandular areas. Superficial glands are present in the lower part of the oesophagus in all the postnatal specimens. The upper groups of superficial glands were found in three of the four specimens examined in serial sections, and in four of the twenty specimens in which only every tenth section was kept. The maximum degree of development of the upper group of the superficial glands found in the present specimens is illustrated in Pl. 4, figs. 22, 23. The deep glands are numerous and occur throughout the length of the oesophagus. In two of the twenty-four postnatal specimens, serous cells and demilunes were found in the deep submucous glands in the upper third of the oesophagus (Pl. 4, figs. 25, 26).

DISCUSSION

Frazer (1931) states that, after differentiation of the oesophagus from the foregut, the oesophageal epithelium proliferates to become stratified. Other authors had claimed that the epithelium persists as a two-layered columnar epithelium up to the 10 mm. stage (Schaffer, 1904), or even to the 25 mm. stage (Schridde, 1904, 1908), but the present work shows that from the 3 mm. stage, which was the earliest in the series, the epithelium is stratified columnar in type and that it remains so until the 16 mm. stage. Between the 23 and 34 mm. stages it is two-layered, after which it proliferates to become a many-layered, low columnar type of epithelium.

Kreuter (1905) maintained that the oesophageal lumen becomes occluded by epithelial proliferation in a manner similar to that which Tandler (1900) had noted in the case of the duodenum in embryos between 30 and 60 days old, and that the oesophageal lumen becomes re-established later by the process of vacuolation. Forssner (1907), Schridde (1908) and Johnson (1910) denied that occlusion occurs, and in the present work, although very marked narrowing at the level of the tracheal bifurcation was noted, actual obliteration of the lumen was not observed.

Forssner (1907) observed vacuoles throughout the whole length of the oesophagus in some specimens, whereas in others they were restricted to the upper and lower parts, while Johnson (1910) found that they are larger and more numerous in the lower half of the oesophagus. Schridde (1908) maintained that true vacuolation does not occur and that the histological appearances may be explained by epithelial bridges, caused by localized epithelial proliferation, linking opposing walls of the oesophagus over localized areas. In the present work, the vacuoles were found to be both larger and more numerous above the level of the tracheal bifurcation than elsewhere, and to increase in size up to the 25 mm. stage; they were present up to the 72 mm. stage, whereas Johnson (1910) found that all traces of vacuolation has disappeared by the 37 mm. stage. These varying observations probably reflect the wide variation that may exist in different specimens. In the present study it was noted that the epithelial partitions separating the vacuoles from the oesophageal lumen are very thin in the case of the larger vacuoles, and Johnson (1910) observed that they rupture, thus throwing the lumina of the vacuoles and the gut into continuity. The significance of the process of vacuolation is not clear, but it has been found to occur in the embryonic oesophagus of the pig (Flint, 1907), rat and rabbit (Johnson, 1910), and hedgehog (Forssner, 1907).

The presence of ciliated cells in the epithelium of the embryonic oesophagus of man was noted by Neumann (1876), Jahrmaeker (1906) and Johnson (1910), but their origin is not clear. The present study suggests that patches of ciliated cells arise multifocally in the middle third of the oesophagus, the patches subsequently enlarging and extending upwards and downwards to form for a time almost a complete epithelial lining to the oesophagus. In these patches, desquamation of cells, with pyknotic nuclei, from the surface of the stratified columnar epithelium, which precedes the ciliated type, suggests that the ciliated cells originate from the deeper cells in the stratified epithelium rather than by direct transformation of the surface columnar cells themselves. In this connexion it may be observed that Jahrmaeker (1906) and Schridde (1908) stated that a basal filamentous process of the ciliated cells connects them to the basement membrane, though such has not been seen in the present work.

In the 130 mm. embryo the stratified columnar ciliated epithelium has, in its turn, begun to be replaced by stratified squamous epithelium, this process also commencing in the middle third of the oesophagus and spreading upwards and downwards. Neumann (1876) considered that the ciliated cells were transformed directly into squamous cells, though Schaffer (1904) maintained that the ciliated cells are desquamated in sheets; this latter process has not been observed in the present work. The last part of the oesophagus to lose its ciliated lining is the upper end, where patches of ciliated cells may exist at, and for a short time after, birth, as was noted by Rector & Connerley (1941). In this region at birth the stratified squamous epithelium is thinner than elsewhere.

The presence in the upper part of the oesophagus of patches of tall columnar epithelium of secretory type was noted in a seven-month embryo by d'Hardivillier (1897) and in a six-month embryo by Schaffer (1904), the latter author claiming that from them develop the superficial oesophageal glands. Lewis (1910) noted such an epithelium in the embryo at both the upper and lower ends of the oesophagus. Boerner-Patzelt (1922) found that many of the columnar cells undergo involution and disappear, and these she distinguished as 'Schleimzellen' in contrast with those in the lateral angles of the oesophagus from which the superficial glands develop and which, following Schaffer (1904) and Schridde (1904), she called 'Magenschleimhautinseln'. In the present series the tall columnar cells make their first appearance at the upper and lower ends of the oesophagus in the 62 mm. embryo. Although the areas occupied by this simple columnar epithelium are of about the same extent in the 62, 72 and 78 mm. embryos, they are reduced in the 90, 110, 130 and 160 mm. embryos, but in the 162 and 172 mm. embryos the areas are as large as those in the 62 mm. embryo. It cannot, therefore, be stated that there is in these stages a progressive reduction in the extent of the areas of simple columnar epithelium and the findings may be attributable to variation in the single specimens. After the 172 mm. stage, the tall columnar epithelium only persists in association with the superficial glands which are developed from it. Between the 90 and 172 mm. stages, groups of shorter columnar cells are present in the upper and lower parts of the oesophagus; they exhibit indistinct cell outlines and blue-staining cytoplasm, and correspond to the 'Schleimzellen' of Boerner-Patzelt. They are not present after the 172 mm. stage. In favour of the division of the columnar cells into two groups, Boerner-Patzelt claimed that there is a difference in their reactions to stains for the demonstration of the presence of mucin, the 'Schleimzellen' giving a positive reaction and the 'Magenschleimhautinseln' a negative one. In the present work no such difference has been found, and the cells of the simple columnar epithelium give a positive reaction, irrespective of their position or appearance.

The superficial oesophageal glands in both the upper and lower ends of the oesophagus have their origin in the tall columnar epithelium, from which they first differentiate and grow as tubules, which extend as far as the muscularis mucosae by the 130 mm. stage. The localization of these glands in the adult corresponds to the localization of the simple tall columnar epithelium in the embryo. As this columnar epithelium is so constant in the embryo, and yet may be absent in the newborn, it appears that on occasions it may completely disappear without giving origin to any superficial glands. In the present work no evidence has been found to indicate the origin of the parietal cells which are present in some of the superficial glands in the newborn and adult. Boerner-Patzelt described them as originating in cells displaced to the periphery of the gland tubules early in their development. Superficial oesophageal glands in the upper part of the oesophagus were found in three of the four postnatal specimens examined in serial section. On the other hand, they were noted in only four out of the twenty postnatal specimens in which only every tenth section was kept, but, as they may be represented by only a single acinus which may be overlooked if any method other than consecutive serial sections be used, their occurrence may be much more frequent than these latter specimens indicate. Schaffer (1904) and Schridde (1904) maintained that they occur in 70 % of adults, whereas Hewlett (1900), Glinski (1903) and Schwalbe & Lubarsch (1905) considered that their frequency of occurrence is much less. Eberth (1897), Taylor (1927) and Rector & Connerley (1941) regarded these glands as heterotopic gastric mucous membrane; the present work shows that they are commonly present in prenatal development and frequently persist into adult life.

Concerning the development of the deep glands, Johnson (1910) mentioned that he had seen them in a 240 mm. embryo, while Healey (1920) was impressed by their absence in a seven-month embryo. None of the embryonic specimens in the present series has deep oesophageal glands, and they are present in only two of the four full-term specimens, in each case being represented by a single gland lobule. There is, however, a solitary well-differentiated mucous gland immediately below the level of the cricoid cartilage in the 110 mm. embryo and in one full-term specimen, but it is suggested that these glands are really pharyngeal in situation, since even in the 90 mm. embryo the mucous glands of the pharynx are well differentiated. Johnson stated that, although there may be no secretory cells in the deep glands at an early stage, such as were described in the pig by Flint (1907), they are usually differentiated in the human at birth. The present work suggests that the deep glands do not appear until the epithelium has become stratified squamous in character, and that they develop as outgrowths from this epithelium. At first these outgrowths are solid, but lumina appear later, and the ducts are lined with an epithelium which is three or four cells thick, while the acini of the glands are lined by a single layer of cubical epithelium. In the early stages of their development the deep glands give a negative reaction for the presence of mucin, in contrast with their strongly positive reaction in the adult. Goetsch (1910) estimated that in the adult there are anything from 60 to 741 deep oesophageal glands. Since the deep glands are so commonly present and widely distributed in the adult, the present work indicates that their development must be mainly postnatal. Schaffer (1897*a*, *b*, 1898), Catania (1926) and Schumacher (1927) stated that the deep glands are solely mucous in type, whereas Eberl-Rothe (1949) maintained that serous glands also occur. In the present work, deep glands possessing both serous acini and demilunes were found among the mucous glands in the upper third of the oesophagus in two of the adult specimens.

SUMMARY

1. From the 3 to the 16 mm. stage the embryonic oesophagus in man is lined by stratified columnar epithelium which in general is three cells deep. Between the 23 and 34 mm. stages it is two-layered; it subsequently proliferates to become many-layered, but occlusion of the oesophagus does not take place, although it may show extreme narrowing at the level of the tracheal bifurcation.

2. Ciliated epithelium appears at the 40 mm. stage, the ciliated cells forming the superficial stratum of the stratified columnar epithelium which comprises an almost complete oesophageal lining up to the 130 mm. stage, when it becomes replaced by stratified squamous epithelium, which first appears in the middle third of the oesophagus. Patches of ciliated epithelium may be present in the upper part of the oesophagus at birth.

3. At the 62 mm. stage, areas of simple columnar epithelium appear in the upper and lower ends of the oesophagus. Some of the columnar cells undergo great increase in height. Later the areas of columnar epithelium become smaller and are replaced by stratified columnar ciliated epithelium.

4. The superficial glands of the oesophagus develop from the tall columnar cells; they first appear at the 130 mm. stage and later undergo further development within the boundaries of the original areas of simple columnar epithelium.

5. Superficial glands may be present in the upper part of the oesophagus in a high proportion of individuals, even though sometimes represented by a single acinus.

6. The deep glands are late in appearance. At birth they are scanty and have not undergone much differentiation; most of their development is postnatal.

7. The presence of demilunes and serous acini in the deep glands is described.

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EXPLANATION OF PLATES

All the specimens illustrated were stained with haematoxylin and eosin, except that in Pl. 2, fig. 11, which was stained for mucin by the periodic acid-Schiff reaction.

Plate 1

Fig. 1. Transverse section of middle of oesophagus of 3 mm. embryo. The epithelium is stratified. ×210.

Fig. 2. Transverse section of middle of oesophagus of 7 mm. embryo. The epithelium has a basal layer of columnar cells. ×300.

Fig. 3. Transverse section of upper third of oesophagus of 11·5 mm. embryo. The epithelium is stratified columnar. ×210.

Fig. 4. Transverse section of middle third of oesophagus of 13 mm. embryo. The epithelium is stratified columnar and a vacuole is seen to the right of the oesophageal lumen. × 300.

Fig. 5. Transverse section of upper third of oesophagus of 23 mm. embryo. The epithelium is two-layered and columnar. × 240.

Fig. 6. Transverse section of upper third of oesophagus of 25 mm. embryo. Two vacuoles are seen, with the lumen of the oesophagus between them. The epithelium on the deep aspect of the larger vacuole (left) is compressed and its nuclei crowded together. × 210.

Fig. 7. Part of the wall of the middle third of the oesophagus of 40 mm. embryo. To the left is stratified columnar epithelium, and to the right stratified columnar ciliated epithelium. The cytoplasm of the ciliated cells stains more darkly than does that of the non-ciliated columnar cells. × 350.

Fig. 8. Part of the wall of the middle third of the oesophagus of 42 mm. embryo. The epithelium is stratified and ciliated, and cells with pyknotic nuclei project above the general level of the epithelium. × 350.

PLATE 2

Fig. 9. Lateral angle of upper third of oesophagus of 62 mm. embryo. To the left and below the epithelium is tall columnar, while to the right it is stratified, with occasional ciliated cells. × 270.

Fig. 10. Transverse section of upper third of oesophagus of 72 mm. embryo. The dorsal wall of the oesophagus is above and the ventral wall below. Note the varying heights of the columnar cells. The epithelium has a uniform thickness. A small vacuole is seen in the left dorsal wall. × 72.

Fig. 11. Transverse section of upper third of oesophagus of 72 mm. embryo. Stained by the periodic acid-Schiff reaction. The columnar cells give a positive reaction. This section is next in the series to that in fig. 10. × 64.

Fig. 12. Higher magnification of part of the section shown in fig. 10. Tall columnar cells with a small vacuole are seen to the left, and stratified columnar ciliated epithelium to the right; between them is a narrow zone of stratified columnar epithelium. × 290.

Fig. 13. Transverse section of upper third of oesophagus of 90 mm. embryo. The columnar cells are increased in height and project above the general level of the epithelium. × 64.

Fig. 14. Right lateral angle of upper third of oesophagus of 130 mm. embryo. To the right a small tubule (*T.*) cut transversely is seen; this is the first stage in the development of the superficial glands. × 255.

PLATE 3

Fig. 15. Transverse section of upper third of oesophagus of 160 mm. embryo. In the left lateral angle, acini of the superficial glands are seen. Note two small groups of columnar cells to the right, adjacent to the ciliated epithelium. × 255.

Fig. 16. Transverse section of upper third of oesophagus of 172 mm. embryo. Note the large areas of tall columnar epithelium and the superficial glands. × 70.

Fig. 17. Lateral angle of the upper third of the oesophagus of 172 mm. embryo. The gland tubules extend down to but do not pierce the developing muscularis mucosae. × 275.

Fig. 18. Lateral angle of upper third of the oesophagus of 210 mm. embryo, showing the further development of the superficial glands. Tall columnar epithelium persists in the region of the glands; elsewhere the epithelium is stratified squamous, but small patches of ciliated epithelium persist. × 42.

Fig. 19. Part of the wall of upper third of oesophagus of 90 mm. embryo. Isolated groups of columnar cells lie among a generally ciliated epithelium. × 250.

Fig. 20. Part of wall of upper third of oesophagus of 130 mm. embryo. The epithelium is ciliated and a small group of columnar cells projects into the oesophageal lumen. × 370.

PLATE 4

Fig. 21. Part of wall of upper third of oesophagus of 130 mm. embryo. The epithelium is ciliated and within its thickness is a cavity lined with ciliated cells. × 530.

Fig. 22. Transverse section of upper third of the oesophagus of adult to show the general characteristics of the superficial glands. × 54.

Fig. 23. Transverse section of upper third of oesophagus of adult to show superficial glands opening into small pits lined with columnar epithelium. × 54.

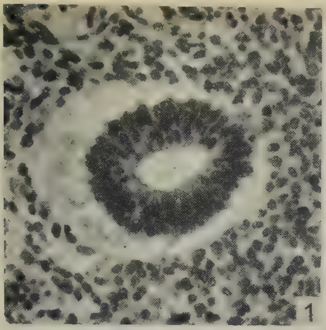
Fig. 24. Transverse section of middle third of the oesophagus of full-term foetus. The downgrowth of a deep gland from the stratified squamous epithelium is shown; there is no differentiation into secretory cells. × 255.

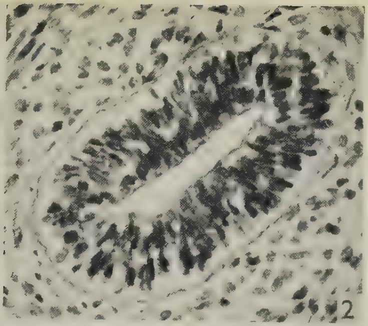
Fig. 25. Transverse section of upper third of oesophagus of adult, showing one deep gland which has both serous and mucous acini. The collections of lymphocytes above and to the left of the gland are common in the vicinity of the deep glands. × 72.

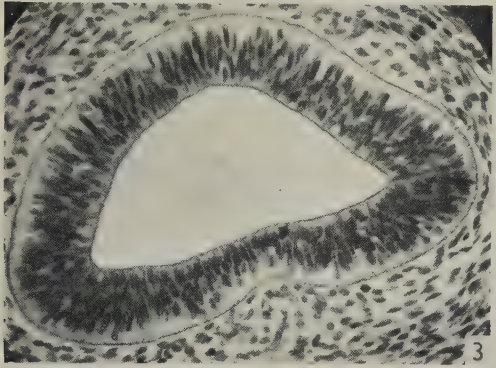
Fig. 26. Transverse section of upper third of oesophagus of adult, showing a deep gland which has mucous and serous acini, and demilunes. × 72.

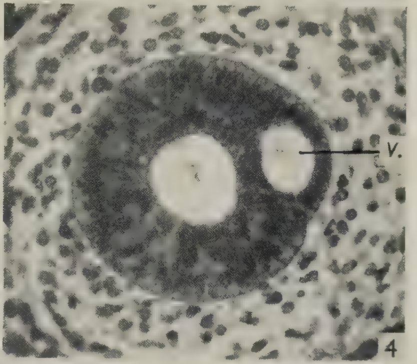
LIST OF ABBREVIATIONS USED IN PLATES

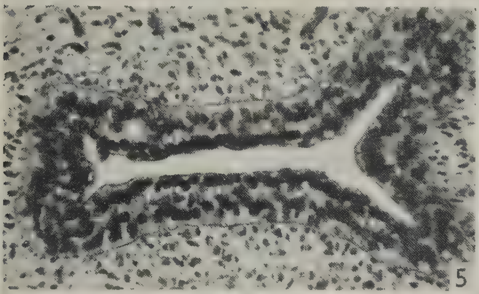
C.	Stratified columnar ciliated epithelium.
D.	Demilune.
L.	Lumen of oesophagus.
M.A.	Mucous acini.
M.M.	Muscularis mucosae.
S.A.	Serous acini.
S.S.	Stratified squamous epithelium.
T.	Developing superficial gland.
V.	Vacuole.

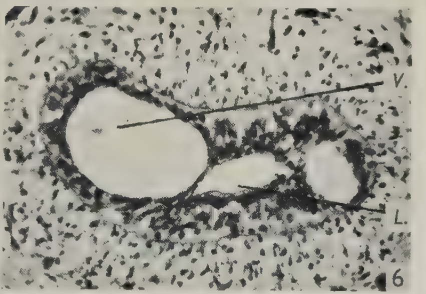


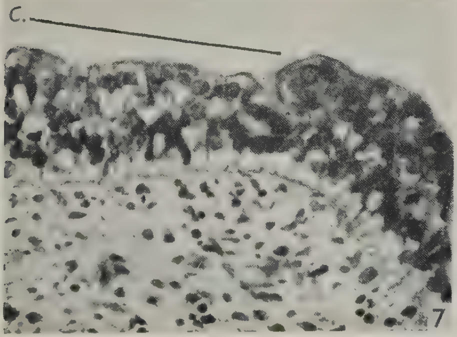


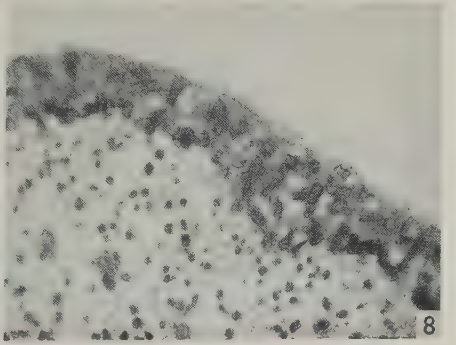












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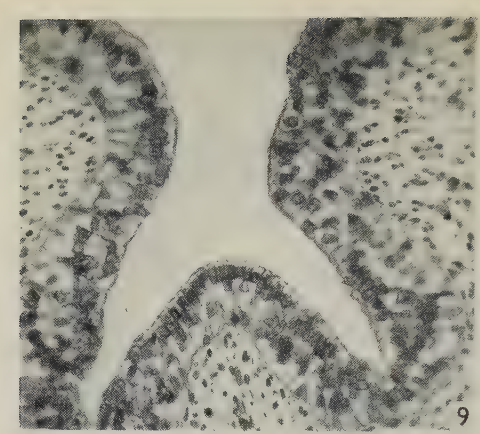
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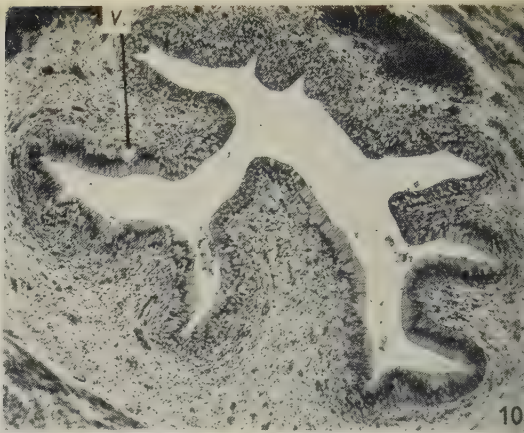

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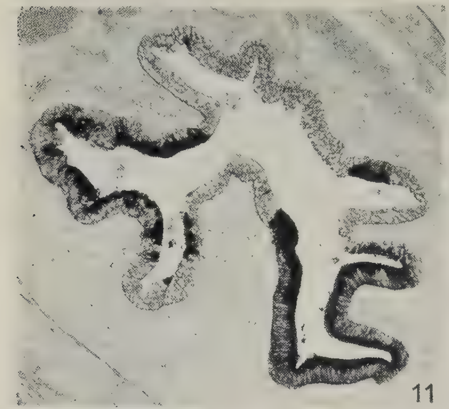
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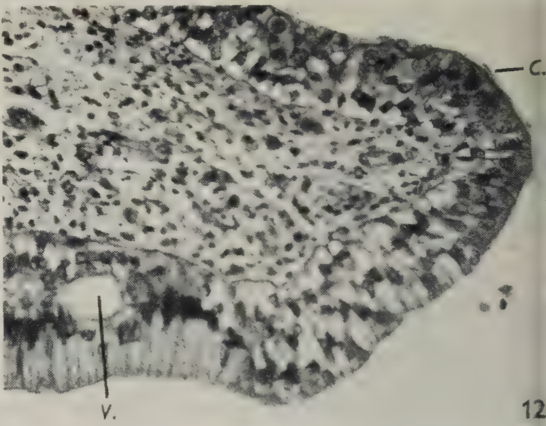

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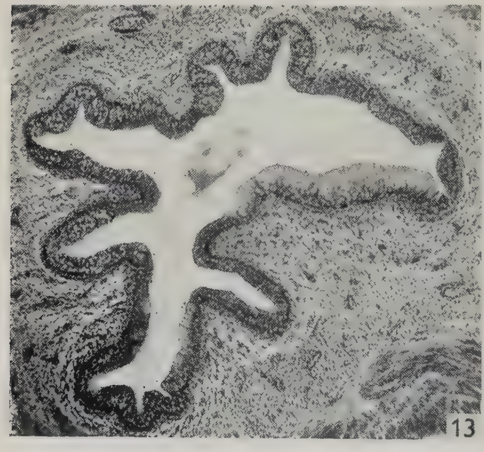
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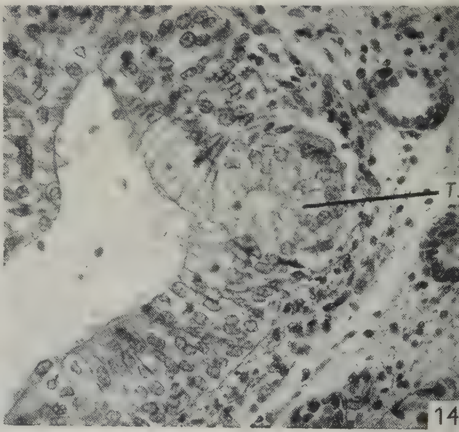

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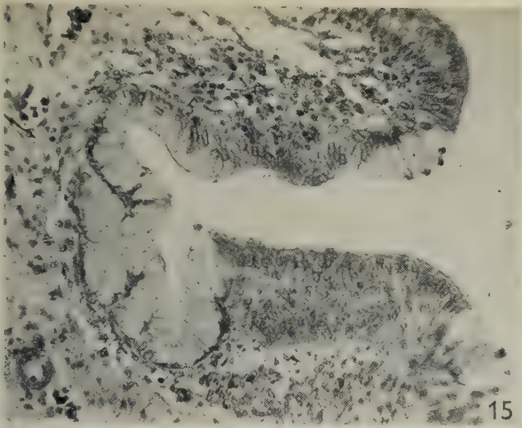
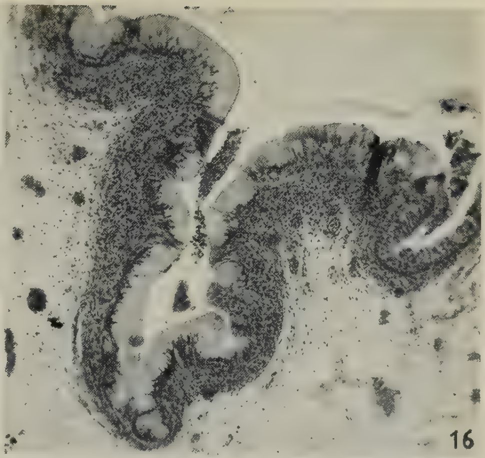
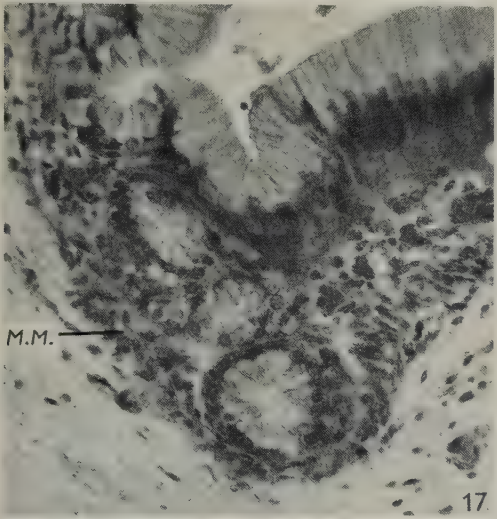
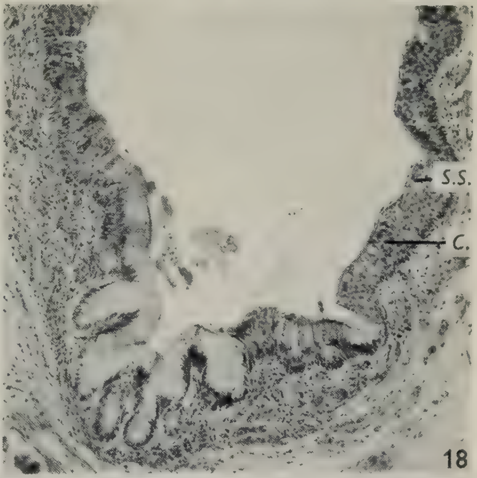
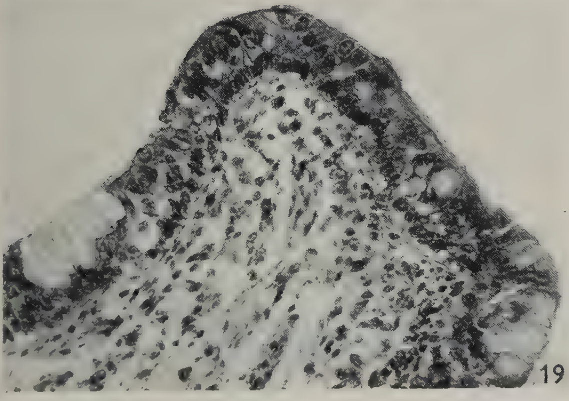
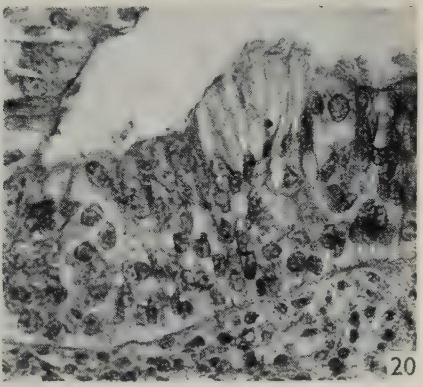




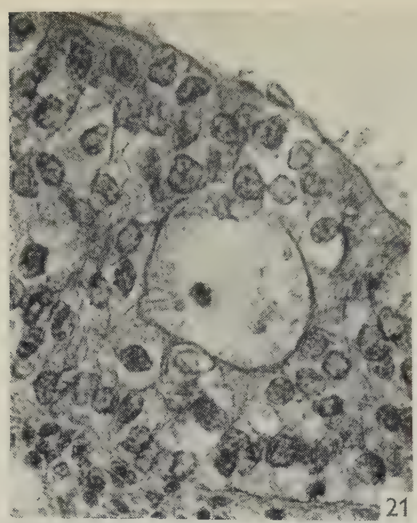








JOHNS—DEVELOPMENTAL CHANGES IN THE OESOPHAGEAL EPITHELIUM IN MAN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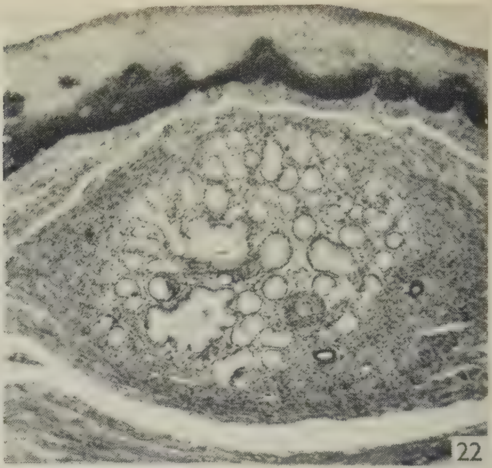
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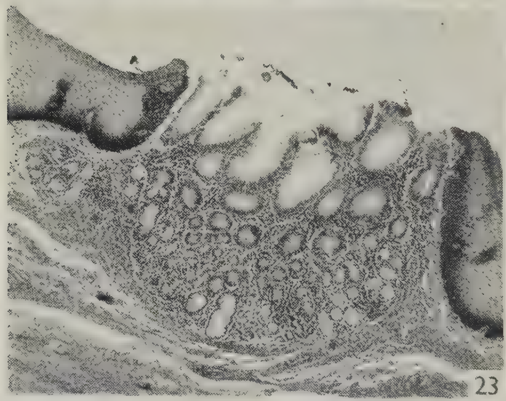
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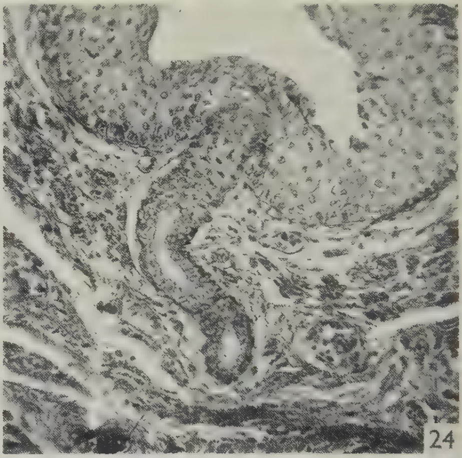
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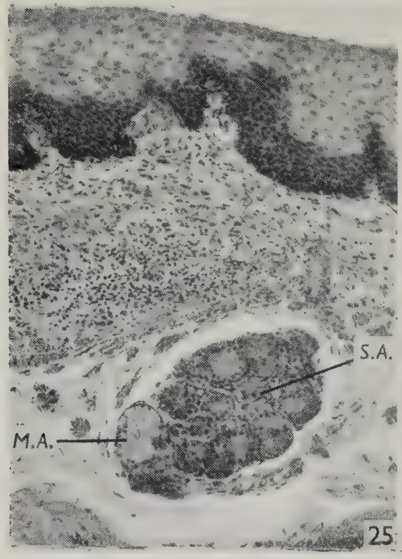

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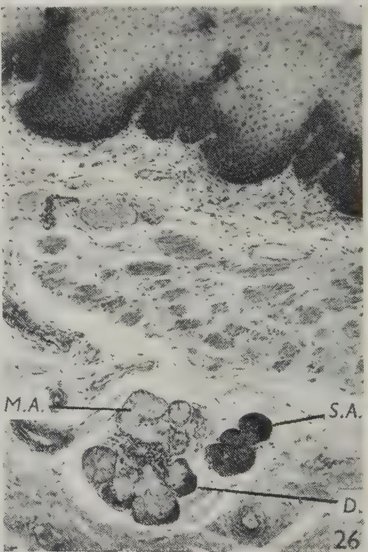

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JOHNS—Developmental changes in the oesophageal epithelium in man

THE EFFECT OF A HIGH TEMPERATURE SHOCK ON EARLY MORPHOGENESIS IN THE CHICK EMBRYO

By ELIZABETH M. DEUCHAR
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INTRODUCTION

The chick embryo has for a long time been popular material for the experimental teratologist. It has been subjected to such varied treatment as shaking, delay of incubation, mechanical injury, ultra-violet and X-ray irradiation, and injection with chemicals. On the whole, these different treatments seem to have produced similar types of abnormalities: possibly the reason why one gains this impression is that writers like Dareste (1891) and Wolff (1948) have painstakingly collected together the results of the multifarious techniques, and have classified and named the monsters produced. The earlier workers were only interested in the anatomy of the abnormal embryos, and did not attempt to explain how they had developed.

One of the simplest experimental procedures to apply to chick embryos under accurately controlled conditions is incubation at abnormal temperatures. Quite a number of workers have used this procedure (see Landauer, 1948, for detailed references), but again have examined only the end results and not the intermediate stages in the development of the abnormalities. For instance, Harman (1922) and Romanoff, Smith & Sullivan (1938) noted a decrease in the viability of chick embryos incubated for long periods at temperatures between 38 and 41° C., but they did not observe the early abnormalities produced. Alsop (1919) made a rather more detailed study, comparing the effects of low and high temperatures on different regions of the central nervous system. She obtained a higher percentage of brain abnormalities with high temperatures (104–108° F.), but more neural tube abnormalities with low temperatures (94–102° F.), applied between 22 and 72 hr., when the neural folds are forming. But the duration of the treatment was too long for it to be possible to tell exactly which morphogenetic processes had been upset.

An investigator who gave more careful attention to the dislocations of morphogenetic movements caused by abnormal temperatures was Grodzinski (1933, 1934). He found that when previously unincubated eggs were kept at 3° C. for periods of up to 72 hr., the presumptive mesoderm underwent centrifugal movements, the reverse of normal, and no streak was formed. In 9–14 hr. embryos subjected to the same treatment, similar centrifugal movements occurred, so that the original streak broadened and became indefinite at its edges, finally dispersing completely. The mesoderm cells appeared less cohesive than in normal primitive streak stages. When incubation was resumed at 37° C., no embryo was formed, although the area vasculosa developed and in some cases spread inwards to cover the area pellucida.

The aim of the present work was to use a treatment slightly less severe than Grodzinski's, and to produce abnormal gastrulation without entirely inhibiting the formation of an embryonic axis. It was hoped to obtain abnormalities which could be interpreted in terms of inhibited or abnormal morphogenetic movements, and so to gain some insight into the significance of the movements which take place normally. It was also of interest to see whether differential effects would be obtained on the various regions of the embryo, as in Alsop's work (1919). A study of these might throw more light on the process of individuation in the chick.

The treatment applied was that of incubation at a high temperature for short, non-lethal periods at different, known stages of gastrulation. Embryos were observed after from 3 to 5 days. A high temperature, rather than a low one, was chosen, because some interesting results have been obtained in amphibian embryos by Brachet (1948, 1949), using high-temperature 'shocks'. He found that gastrulation was arrested, while the synthesis of ribonucleoproteins also stopped. It would be interesting, therefore, to see whether comparable effects were obtained in chick embryos: i.e. whether it is a general phenomenon for invagination to be arrested by a high-temperature shock. In the present work, no attempt has so far been made to determine ribonucleoprotein concentrations in either normal or treated embryos: only the morphogenetic effects of the high temperature have been studied.

MATERIAL AND METHODS

Fertilized eggs obtained from the Poultry Research Centre, Edinburgh, were incubated in thermostatically controlled incubators: one, for the controls, at 37·5° C., and the other, for applying the high-temperature shock, at 45·5° C. The eggs were spaced out on zinc shelves which were covered with a layer of cotton-wool. The atmospheric humidity in the incubators was maintained at a suitable level by keeping vessels of water in them. Under these conditions, the controls developed normally.

After incubation for a suitable period at 37·5° C., each egg was opened, using aseptic precautions, to observe the developmental stage reached by the embryo. A small window about 1·5 cm. square was made in the shell with a fine hacksaw blade, and the square of shell removed. When the shell membranes were deflected, the embryo could be observed. The piece of shell was then replaced, and sealed down with paraffin wax. In fifty-one cases, a sterile glass cover-slip was substituted for the shell, so that development could be observed through it each day.

It was necessary to make these observations before each experiment, since there is considerable variation in the stage of development reached by individual chick embryos of the same incubation age. This is because eggs ovulated after 4 p.m. undergo development in the oviduct all night before they are laid, whereas those ovulated during the day are laid with much less delay (Shearer, 1924; Peter, 1938). The shape of the area pellucida was always clearly distinguishable, and although no micrometer measurements were made, the relative lengths of the streak and area pellucida could be compared by eye with sufficient accuracy for each 12, 16 or 19 hr. embryo to be relegated quite certainly to one of the three following stages (cf. Waddington, 1932):

(*a*) Short primitive streak (*S*) stage, with the area pellucida circular and the streak extending for not more than half its length.

(*b*) Medium primitive streak (*M*) stage, with the area pellucida elliptical and the streak extending for two-thirds of its length. At this stage (Pasteels, 1937, 1940), both prechordal and lateral plate mesoderm have invaginated.

(*c*) Definitive streak (*L*) stage, with a pear-shaped area pellucida and a primitive streak of more than two-thirds of its length. The notochord mesoderm (head process) has just begun to invaginate at this stage (Pasteels, 1937).

Since on these criteria nearly all the embryos in each age group were found to be at the same stage of development, it was considered legitimate to group the results according to incubation age at the time of exposure to the high temperature.

The eggs were allowed to cool to room temperature after they had been opened. For the experiments, they were re-incubated for either 3 or 5 hr. at 45·5° C., then returned to the 37·5° C. incubator for 2 or 3 days. Controls were kept continuously at 37·5° C., after they had been observed at the same stage as the experimentals.

Embryos were finally fixed in Bouin's fixative. All of them were examined as whole mounts, stained with Mayer's Carmalum. Twenty-four were later sectioned, after they had been photographed whole. Sections were stained with Ehrlich's haematoxylin.

EXPERIMENTS

Embryos of five different ages were exposed to the brief 'shock' treatment at 45·5° C.

In series 1, 19 hr. embryos were treated in order to determine the effect of high temperature on morphogenetic processes immediately following gastrulation. In series 2 and 3, 16 and 12 hr. embryos were treated, respectively. Finally, embryos were treated prior to streak formation. It was not considered valuable to treat stages between 0 and 7 hr., since according to Patten (1929) from 3 to 5 hr. should be allowed before the whole egg can be considered to have attained the temperature of the incubator. So in series 4, 7 hr. embryos were treated, while in series 5, embryos were treated prior to incubation.

Series 1. 19 *hour embryos*

After incubation for 19 hr. under the present conditions the primitive streak extended for at least two-thirds of the length of the area pellucida in most embryos. Reference to Pasteels's maps of presumptive areas on the chick blastoderm (1940) shows that the axial mesoderm should be nearly if not completely invaginated at this stage.

(*a*) 3-*hour treatment*

In preliminary experiments, nine embryos were exposed to the temperature of 45·5° C. for 3 hr. Four of them were examined after a further 22 hr. incubation at 37·5° C., and the other five after 42 hr. further incubation.

Four controls were incubated continuously at 37·5° C. Two of these were fixed after 40 hr., and two after 60 hr., to correspond in age with the experimentals.

Results (see Table 1). Eight of the treated embryos had developed normally to stages with completed cranial flexure and beating heart. The remaining one had been destroyed by rupture of the vitelline membrane. One of the controls had also been destroyed in this way, probably owing to damage incurred when the egg was opened, either for the preliminary observations or just before fixation. The other three controls were normal.

It is clear from these results that a 3 hr. exposure to the high temperature had no effect on embryos of this stage. It was decided to prolong the exposure, in case 3 hr. had been too short a time for the eggs to warm up to 45·5° C.

(*b*) *5-hour treatment*

Fifteen embryos were exposed to 45·5° C. for 5 hr. In all of them the streak extended for more than two-thirds of the length of the area pellucida at the time of treatment. They were examined after a further 70 hr. incubation at 37·5° C.

Results (Table 1). Nine embryos were abnormal. Three of them were necrotic, showing only a small whitish mass of tissue in the centre of the area pellucida. Two embryos had become peculiarly distorted. One of these, C 87 (Pl. 1, fig. 1), was particularly interesting because the heart lay anterior to the other axial tissues, which were considerably shortened, and were enclosed in a pear-shaped vesicle formed by the amnion. The neural folds were distorted, and were widely separate in the mid-line, indicating that neurulation had been incomplete.

The second of these distorted embryos (Pl. 1, fig. 2) was also shortened, but not as much as C 87. In this, the neural folds, although bent, were parallel to one another in the mid-line. The heart lay in its normal position.

Table 1. *Effect of high temperature on* 19 *hr. embryos*

Duration of exposure at 45·5° C.	Total treated		Vitelline membrane broken		Arrested: necrotic		Arrested: some differentiation		Dwarf		Development delayed		Normal	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
3 hr.	9	100	1	11	0	0	0	0	0	0	0	0	8	89
5 hr.	15	100	0	0	3	20	2	13	2	13	2	13	6	40
Controls	4	100	1	25	0	0	0	0	0	0	0	0	3	75

There were also two dwarf embryos. These had not been so severely affected by the high temperature, for they had neurulated normally and showed a normal cranial flexure. In one, it was only the posterior trunk which was shortened. This embryo was heterotaxic, with its head and anterior trunk bent to the left and with the heart on the left side. But as there were three heterotaxic embryos among the controls, this condition cannot be taken to have resulted from the high-temperature shock. Other workers have also noted instances of heterotaxia among normally incubated embryos.

In the two 'delayed' embryos (Table 1), flexure had only just begun, whereas normally this process is complete by 70 hr., the embryo having already rotated to lie on its left side. In one of the delayed embryos no heart had yet developed.

To summarize the abnormalities caused by exposure to high temperature at the 19 hr. stage: three necrotic embryos; two distorted, shortened embryos in which neurulation was incomplete; two slightly shortened (dwarf) embryos, and two in which development had been delayed. In all, 60 % of the embryos given a 5 hr. treatment were abnormal.

Series 2. 16-*hour embryos*

In all of these the area pellucida was circular or elliptical, with the streak extending for one-half to two-thirds of its length.

(*a*) 3-*hour treatment*

Thirty-four embryos were exposed to 45·5° C. for 3 hr., and fourteen controls were incubated at 37·5° C. for the same period. All were fixed and examined after 60 hr. further incubation.

Results (Table 2). In ten of the treated embryos the vitelline membrane had ruptured. (This also occurred in one of the controls.) Seven were abnormal as a result of the treatment.

In one of the abnormal embryos the streak tissue had been killed and was disintegrating. Another showed a small, rounded mass of mesoderm, the remains of the streak, in the centre of the area pellucida. This tissue was found on sectioning to be undifferentiated.

In the other five abnormal embryos some differentiation was visible. Table 3 gives details of the tissues present in them. For instance, A71 and A72 (see table) contained neural tube, notochord and somites, but no brain, and there was no mesoderm anterior to the heart. In A71 all the embryonic tissue was collected as a short, pear-shaped mass, but A72 had a normally extended posterior streak.

In embryos A63 and A89, the neural tube, notochord and somites were present for a short distance, but there was no heart. An undifferentiated mass of mesoderm lay at the anterior end of the streak in both these embryos.

Table 2. *Effect of high temperature on 16 hr. embryos*

Duration of exposure	Total treated		Vitelline membrane broken		Gastrulation arrested: necrotic		Arrested: some differentiation		Dwarf		Development delayed		Normal	
at 45·5° C.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
3 hr.	34	100	10	29·4	2	5·9	5	14·7	0	0	1	3·0	16	47·1
5 hr.	31	100	0	0	15	48·4	3	9·7	4	12·9	0	0	9	29·0
Controls	14	100	1	7·1	1	7·1	0	0	0	0	0	0	12	85·8

A62 was the only one of the seven abnormal embryos which possessed a brain. Here, the neural tube was very short, there were only two pairs of somites, and the posterior axial mesoderm was undifferentiated. So evidently only the anterior tissues had been able to develop normally.

There were two distorted embryos. A23 had bends in the mid-brain and anterior trunk regions, and therefore appeared shorter than normal. In A81 there was less apparent shortening, but the cranial flexure was towards the left side, so that the whole axis was S-shaped. The heart also lay on the left side.

In one embryo development had been delayed. It showed incomplete neurulation, whereas in controls this process was complete, the brain was clearly defined, and cranial flexure had taken place.

Sixteen out of the thirty-four treated embryos, and twelve out of fourteen controls, developed normally.

(*b*) *5-hour treatment*

Thirty-one embryos were subjected to treatment at 45·5° C. for 5 hr. They were examined after a total incubation time of 90 hr. (At this stage the normal embryo shows completed flexure, and has gill-clefts and a beating heart.)

Results (Table 2). Fifteen embryos were killed by the treatment: a much higher percentage than among those treated for only 3 hr. In all the dead embryos the streak had degenerated into an irregular, white mass of necrotic cells. Nine of them, however, showed a well-developed area vaculosa, with the beginnings of capillary blood vessels.

Three embryos were abnormal. They were examined as whole mounts. In two of them the neural folds were shorter than normal, but there was a definite brain, although this was not differentiated into its three main regions. One embryo was omphalocephalous (i.e. with the heart lying anterior to the brain, as described in C87 on p. 446).

The third abnormal embryo contained irregularly arranged, dead-looking mesoderm tissue within the amniotic vesicle. No nervous system had been induced.

There were four dwarf embryos, one of which was extremely shortened in both head and trunk regions. The other three were only slightly shortened, but had died before the 90 hr. stage. One of them was heterotaxic.

Table 3

Embryo no.	Brain	Neural tube	Neural plate	Notochord	Somites	Heart	Foregut	Undifferentiated mesoderm
A 62	+	+	−	+	+	+	−	−
A 63	−	+	−	+	+	+	+	−
A 71	−	+	−	+	+	+	+	+
A 72	−	+	−	+	+	+	+	−
A 89	−	−	+	+	+	−	+	−

In summary of the results in this series, it can be said that 16 hr. embryos showed a much higher susceptibility to the high temperature than did 19 hr. embryos. The effects of the 5 hr. treatment were the most severe: abnormalities arose in over 70 % of the embryos (Table 2), and many were killed. The six embryos which did survive were shortened both anteriorly and posteriorly, since the development of both head and trunk had suffered some inhibition. The partial embryos resulting from a 3 hr. treatment, however, had been inhibited in either head or trunk regions, but not both: except for A62, it was the head which had been affected.

Series 3. 12-*hour embryos*

Ten of the forty-nine embryos used in this series had a streak of two-thirds of the length of the area pellucida, indicating that the prechordal mesoderm had already invaginated. But the majority of them (twenty-two) had a short streak, extending for only half the length of the area pellucida. Pasteels's maps (1937, 1940) show the presumptive mesoderm to be disposed on the surface of the circular blastoderm at this stage. In addition, there were seventeen embryos in which the streak was not visible.

Most of these embryos were observed daily during the experiment through glass cover-slips sealed over the window in the shell. They were fixed, with nine controls, after 104 hr. incubation.

(*a*) 3-*hour treatment*

Eight embryos were exposed to 45·5° C. for 3 hr.

Results (Table 4). Five embryos developed normally. Of the three others, one had ruptured the vitelline membrane, so could not be observed.

There was only one embryo in which morphogenesis had certainly been affected by the treatment: this was the dwarf specimen. Its head was especially reduced in size, so that the fore-brain lay on a level with the ventricle of the heart. The cranial flexure was very slight.

There was one case of duplicitas posterior: the only one observed throughout this work. Although this condition could have resulted from abnormal invagination, one must be cautious in attributing it to the high-temperature treatment, since other workers have noticed this abnormality among their controls. Eight of the nine controls in this series developed normally.

(*b*) *5-hour treatment*

Forty-one embryos were exposed to 45·5° C. for 5 hr.

Results (Table 4). Twenty embryos had been killed by the treatment. Eight of them showed some necrotic streak tissue (cf. Pl. 1, fig. 3). In all of these embryos the peripheral part of the area vasculosa had developed.

There were two partial embryos in which the heart was the only recognizable organ. In three others, where some nervous tissue was present, the heart was the most prominent and well-developed organ.

Table 5 gives details of the tissues observed in nine embryos in which some axial development had taken place. Certain regions of the axial mesoderm were absent, or much reduced: these were regions which, according to observations of the streak length at the 12 hr. stage in individual cases, would have been about to invaginate when the high-temperature treatment was applied. For instance, four embryos had shown a streak extending for two-thirds of the length of the area pellucida, indicating that head, but not trunk axial mesoderm had invaginated already. At 104 hr., these had all formed a normal brain, but the trunk was greatly reduced. One of these embryos is shown in Pl. 1, fig. 4. The trunk region has become distorted to lie transversely. Both brain and heart are well-differentiated.

In contrast to these four embryos were three which had shown a streak occupying only one-half the area pellucida length at the 12 hr. stage. After the experiment, these had no head region, although trunk tissues were present. Clearly it was the head mesoderm, not yet invaginated from the short streak at 12 hr., which had been affected by the treatment in these embryos. Pl. 1, fig. 5, shows one of them, in which a mass of presumptive head mesoderm has collected at the node, and the neural folds are distorted into a diamond shape outlining the mesoderm mass. There was one other headless embryo which bore some resemblance to C87 in series 1. All the mesoderm had collected as a broad mass posterior to the original position of the node, and had induced a broad neural plate above it. In section (Pl. 1, fig. 6) the notochord was seen to be double beneath the broadest part of the neural plate.

Two further embryos which had shown a half-length streak at the 12 hr. stage had differentiated after 104 hr., but were shortened in the head region, indicating that again the anterior mesoderm had been most affected. They are classed as 'dwarf' in Table 4.

An interesting abnormality arose in an embryo in which the streak had not yet formed at 12 hr. In this, the invagination of anterior and posterior mesoderm was not inhibited, but the whole axis was distorted into an S-shape. The neural folds were of normal length in spite of their distortion, and a brain vesicle had formed at their anterior end. It is difficult to say just what abnormal morphogenetic movements led to the assumption of this S-shape, but the interesting point is that some

Table 4. *Effect of high temperature on 12 hr. embryos*

Duration of exposure to 45·5° C.	Total treated		Vitelline membrane broken		Arrested and necrotic		Arrested: axial differentiation		Arrested: heart only		Dwarf		Development delayed		Normal	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
3 hr.	8	100	1	12·5	0	0	1	12·5	0	0	1	12·5	0	0	5	62·5
5 hr.	41	100	1	2·5	20	48·8	9	21·5	2	4·9	2	4·9	0	0	7	17·1
Controls	9	100	1	11·1	0	0	0	0	0	0	0	0	0	0	8	88·9

Table 5. *Differentiation observed in histological sections of arrested 12 hr. embryos*

Embryo no.	Streak length: area pellucida length	Brain	Optic and otic vesicles	Neural tube	Neural plate	Notochord	Somites	Lateral mesoderm	Heart	Undifferentiated mesoderm	Foregut	Area vasculosa
B 86	1	+	+	+	–	+	+	+	+	–	+	+
B 54	$\frac{2}{3}$	+	–	+	+	+	+	–	+	–	–	+
B 70	$\frac{2}{3}$	+	+	–	–	+	+	–	+	+	+	+
B 44	$\frac{1}{2}$	–	–	–	–	+	+	–	–	+	–	+
B 75	$\frac{1}{2}$	–	–	+	+	+*	–	–	+	–	+	+
B 34	$\frac{1}{2}$	–	–	–	+	–	–	–	–	+	–	–
B 61	$\frac{1}{4}$	–	–	+	+	+	+	–	+	–	+	+
B 39	?	–	–	+*	–	+*	–	–	+	+	–	+
B 74	–	(+)	–	+	+	+	+	–	+	–	+	+

* Double.

of the 7 hr. embryos described in the next series became distorted in this way after exposure to the high temperature. These also had no streak at the time of treatment.

There were two further abnormalities in this series. One embryo had formed two axes in alignment, which were orientated in opposition, with their anterior ends (distinguishable by a slight broadening of the neural tube) meeting in the middle of the blastoderm. It is difficult to see what effect of the high temperature may have been responsible for this 'twinning': more probably the blastoderm was damaged accidentally at the 12 hr. stage. Lutz (1948) has obtained similar twin embryos, orientated head-to-head, by making a transverse wound in the chick blastoderm before incubation.

The remaining abnormal embryo possessed no neural tissue, although a notochord and somites had formed. The reason for this failure of induction was not apparent.

Only seven out of the forty-one embryos treated in this section developed normally: a much lower percentage than among those treated at later stages.

The much greater effect of 5 hr. exposure to 45·5° C., as compared with an exposure of only 3 hr., is most noticeable in this series. Whereas only one out of eight embryos treated in section (*a*) showed an abnormality which could be directly attributed to the high temperature, over 80 % of those in section (*b*) had been affected. These included twenty killed outright, and nine in which the abnormalities produced could be correlated with the stage of gastrulation reached after 12 hr. incubation. It is apparent that each region of the mesoderm is most susceptible to the high temperature at the time when it is invaginating.

Series 4. *7-hour embryos*

After 7 hr. incubation all that could be seen in most embryos was an irregular, ill-defined mass of cells in the centre of the blastoderm. There was no distinct area pellucida, and no signs of a primitive streak. In five embryos, the blastoderm was uniformly opaque, with no visible differentiation.

All forty-two embryos in this series were given 5 hr. exposure to the temperature of 45·5° C. They were examined after incubation for a further 42 hr. at 37·5° C.

Results (Table 6). Thirteen embryos had been killed. In these, the mesoderm had converged towards the mid-line, but no invagination or subsequent development had taken place. The tissue had collected as a round knob in the centre of twelve of these blastoderms, while in the other one it had formed a short streak.

In six embryos some abnormal development had occurred. They were examined as whole mounts. In four of them the streak and neural folds were distorted into an S-shape like those of B74 described in series 3(*b*). In the remaining two, no differentiated tissues were present except the heart.

There were six dwarf embryos, in which both head and trunk were equally shortened. The remaining sixteen embryos were normal in appearance.

As Table 6 shows, there was a considerable percentage of abnormalities among the controls in this series. It is possible that they had been affected by being cooled down to room temperature after the eggs had been opened for the preliminary observations. But the controls in other series had not been adversely affected by this cooling, and it was not evident why 7 hr. embryos should have been especially susceptible. According to the experimental data, high temperature affected them less than either 12 or 16 hr. embryos: only 61 % of the embryos in this series were abnormal, after a 5 hr. treatment. The converging movements of the mesoderm had not been inhibited, although in sixteen cases the streak which formed had been killed or distorted. In two embryos, the heart mesoderm had invaginated normally.

Series 5. Unincubated embryos

These embryos were not observed before exposing them to the high temperatures, since it is not easy to distinguish the different stages of development prior to incubation. The evidence (Patten, 1929) is that a variable amount of cleavage takes place before the egg is laid.

Twenty-nine unincubated eggs were treated at 45·5° C. for 5 hr., then incubated for a further 70 hr. at 37·5° C.

Results

Twenty-eight embryos developed normally. Two of these were retarded, however, having only just begun flexure at the 70 hr. stage. There was also one very slightly shortened embryo. The single mortality in this series was due to rupture of the vitelline membrane.

It can be concluded that the unincubated embryos were practically unaffected by the high-temperature treatment.

DISCUSSION

These experiments can only be considered to be a very preliminary investigation into the effects of high temperature on morphogenetic processes in the chick. A more detailed analysis of this problem would require much more exact measurements of the stage of gastrulation reached in individual embryos at the time of treatment. It would also be desirable to use conditions in which higher percentages of abnormalities were produced, so that a series of stages in the development of each type of abnormality could be observed in fixed preparations. But the results obtained in this work have at least brought to light two facts: that a brief exposure to high temperature at the gastrula stage will cause abnormalities to arise, and that many of these abnormalities appear to be due to inhibition of invagination. The last fact is the more significant, because it is known that high temperature inhibits gastrulation in Amphibia (Brachet, 1949).

The interpretation of how the abnormalities arose has had to be largely hypothetical, since embryos were not observed until late stages. But a general survey of the types and frequencies of abnormalities in each age group supports the view that gastrulation was either dislocated or entirely inhibited during the period of treatment. Table 7 summarizes the effect of high temperature on embryos of all ages used. It is immediately obvious that gastrulation stages were most affected; few abnormalities were obtained in 19 hr. embryos, where gastrulation was complete, and none in unincubated embryos where it had not yet begun. Further, when the types of abnormalities imputed to effects on gastrulation were examined in more detail, it was noticed that those regions of the mesoderm which would have been about to invaginate at the time when treatment was begun, were absent altogether or had collected as an undifferentiated mass in the position originally occupied by the node. Reference to maps of presumptive areas on the chick blastoderm (Pasteels, 1937;

Table 6. *Effect of 5 hr. exposure to high temperature on 7 hr. embryos*

	Total treated		Vitelline membrane broken		Arrested and necrotic		Arrested: some differentiation		Dwarf		Development delayed		Normal	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Experiments	42	100	1	4·8	13	31·0	6	12·9	6	12·9	0	0	16	38·1
Controls	13	100	0	0	4	31·4	1	7·7	1	7·7	0	0	7	53·8

Table 7. *Percentages of various abnormalities obtained in different embryonic stages treated*

Stage	Duration of treatment (hr.)	All abnormalities	All gastrulation abnormalities	Necrosis	No axial tissues: (i.e. heart present only)	No head	No trunk	Distortion	Dwarfism	Delayed development
7 hr.	5	56·8	9·6	31·0	9·6	.	.	19·2	12·9	.
12 hr.	3	25·0	(12·5)*	.	.	.	.	12·5	12·5	.
	5	80·1	26·4	48·8	4·9	7·5	9·6	2·5	4·9	.
16 hr.	3	23·9	15·0	5·9	.	3·0	12·0	.	.	3·0
	5	61·0	9·7	48·4	.	.	.	9·7	12·9	.
19 hr.	3	.	.	.	.	.	.	.	.	.
	5	59·6	.	20·0	.	.	.	13·2	13·2	13·2

* Duplicitas posterior—possibly arose spontaneously, or after mechanical injury.

Waddington, 1952) confirmed this observation. It was noticeable in 12 hr. embryos, where the streak-length at the time of treatment was most variable.

At the 7 hr. stage, the morphogenetic movements taking place consist of a convergence of mesoderm tissues towards the posterior and the mid-line of the blastoderm. All the abnormal embryos in this series gave evidence that the mesoderm had been able to converge normally to the mid-line: that it was later morphogenesis which had gone awry. Even the necrotic blastoderms showed a mass of mesoderm collected in their centre. One may also recollect that in one abnormal 16 hr. embryo and in some 12 hr. embryos (Pl. 1, figs. 3 and 5), large collections of mesoderm were present in the original node region suggesting that all the mesoderm had converged into this position before its further movements stopped.

One abnormality which was characteristic of 7 hr. embryos and did not arise in primitive streak stages subjected to the high temperature, was the S-shaped distortion of the streak and neural folds. There are two possible ways of accounting for it. One is to suggest that during streak formation the convergent movements of the two sides became asynchronous, so that instead of the two opposing streams meeting at the same level in the mid-line, they streamed in alternately in a continuous sequence, first one side, then the other, being able to overshoot the mid-line slightly, and resulting in an undulating streak. The second possibility would be that, owing to some inhibition of anterior-posterior stretching after the axial mesoderm had reached its definitive position, the embryo had become telescoped as it grew in length. This process would, however, occur long after the high-temperature treatment had finished, and it is difficult to see what was the primary effect of the treatment.

The striking absence of abnormalities in embryos exposed to 45·5° C. prior to incubation (series 5) deserves further comment. Other workers have observed that the unincubated chick embryo is peculiarly resistant to temperature change. For instance, Romanoff (1936) reports that unincubated hens' eggs can be refrigerated without harm; a treatment which is fatal when incubation has been started. Pritsker (1940) even claims that slight cooling prior to incubation has a *beneficial* effect. Since it is known that between laying and incubation development is suspended, or proceeds only very slowly (Olsen, 1942), one might suggest that the resistance of these embryos to changes in temperature is due to their low rate of metabolism, and the virtual absence of morphogenetic movements in them.

These considerations point to what is already clear from Table 7, namely that stages characterized by major morphogenetic movements are especially sensitive to temperature change. It has already been remarked that the invaginating cells appear always to have been affected more than other tissues. Their special susceptibility may be due to the 'de-epithelisation process' (Waddington & Taylor, 1937) which they undergo, causing them to separate from one another and to become amoeboid. In this condition, their cell membrane would more easily be affected by surface changes than when they were part of a continuous epithelium. Grodzinski (1934) reported a decreased cohesiveness in the primitive streak cells after treatment of embryos with low temperatures, so that evidently the surface properties can be altered by temperature change. It was not clear in the present work if the mesoderm cells became either more or less cohesive, but their tendency to collect at the node instead of spreading in an anterior-posterior direction might be attributed to an increased cohesion between them. It is, however, probable that the behaviour of the invaginating cells is also governed by tensions in the area pellucida, since during gastrulation all the embryonic tissues move in conjunction with one another: starting as a continuous surface sheet, they converge in sequence until the moment of their invagination. For this reason any abnormal movement in one part of the tissue would affect the movements of the rest. A primary effect of the high temperature on the surface tissues might therefore produce abnormalities in the tissues due to invaginate.

On the other hand, observations on the necrotic embryos emphasize the high susceptibility of the invaginating cells. In all of them, the obviously dead region consisted of a small patch in the centre of the blastoderm where the streak should be. The rest of the area pellucida remained intact, and often differentiated as area vasculosa at its periphery.

It has already been remarked (p. 454) that in some of the treated embryos the invaginated mesoderm had accumulated at or near the original position of the node. This suggests that one effect of the high temperature was to inhibit longitudinal extension of the tissues. The suggestion is further supported by the occurrence of dwarf embryos which, although they had differentiated normally, were reduced in length.

Some other features of the abnormal embryos are worth considering. There were several instances, especially in series 2 and 3, where a part of the presumptive neural plate had not been underlain by mesoderm because invagination had been arrested. Not one of these showed any ability of the presumptive neural plate to self-differentiate: only those parts which had been underlain by mesoderm became nervous tissue. Further, there was a remarkable correlation between the shape of the neural plate and that of the mesoderm. Examples of this were described in series 3, and two are shown in Pl. 1, figs. 5 and 6. In the first of these the neural folds outline the diamond-shaped mass of mesoderm; in the second they take on an S-shape, and in the third the neural plate is broad, especially in the region where the notochord is double (Pl. 1, fig. 6). It is quite clear that the position and shape of the mesoderm has controlled the differentiation of the nervous system. Grünwald (1935) appears to have obtained similar results. He describes 'platyneurous' embryos with abnormally wide, short neural plates, and with mesoderm in which there was often a supernumerary notochord or somites lying lateral to the primary tissues. He argued that this abnormality was due to an inducing action exerted on the mesoderm by the neural plate. It would be equally logical, however, and much more in accordance with current ideas on organizer action, to argue that the supernumerary mesoderm induced the extra wide neural plate. The only known cases of induction by ectoderm in the chick are those of Waddington & Taylor (1937), who grafted pieces of ectoderm into the node region. These grafts only acquired inducing powers if they invaginated with the mesoderm and underwent a 'de-epithelisation process'. There are therefore no grounds for assuming that induction normally proceeds from ectoderm to mesoderm. Moreover, mesoderm is able to differentiate without the presence of a neural plate above it, for a case was described in series 3 where a notochord and somites were present, although there was no nervous tissue.

The results of the present experiments confirm the view that in the chick, normal induction cannot occur without invagination, for whenever any part of the mesoderm failed to invaginate, it neither differentiated nor induced. Instead, it usually collected as an undifferentiated mass which must have impeded morphogenetic movements in the tissues immediately adjacent to it. This blocking effect by the uninvaginated mesoderm was probably responsible for the failure of competent lateral tissues to form a 'substitute' embryonic axis like those arising in Waddington's (1932) and in Abercrombie's (1950) experiments.

It is clear, then, that in the chick, whether undergoing normal development or regulation following injury, normal morphogenetic movements of the competent tissues are essential to the formation of a normally differentiated embryonic axis. Invagination, and longitudinal extension, are two important morphogenetic movements, and both of these are susceptible to high-temperature treatment. It is desirable that any further investigations of the effects of high temperature should include detailed studies on the physico-chemical properties of the invaginating cells. This would bring chick embryology more into line with the recent work on amphibian embryos, where interest is now centred on the properties of individual cells rather than of coherent tissues.

SUMMARY

Embryos of five different stages between 0 and 19 hr. incubation were exposed to a high-temperature 'shock' at 45·5° C. for either 3 or 5 hr. After further incubation at the normal temperature (37·5° C.) they were examined for effects on regional differentiation in the axial tissues.

1. 19 hr. embryos (head-process beginning to form) were unaffected by 3 hr. exposure to the high temperature, but 5 hr. exposure killed 20 % and produced abnormalities in 39·6 % (Table 1). The abnormalities included two distorted, shortened embryos in which neurulation was incomplete: two dwarfs, and two retarded in development. These effects could all be explained as due to an inhibition of late stages of gastrulation.

2. With 16 hr. embryos (prechordal mesoderm partially invaginated), a 3 hr. exposure to high temperature killed 5·9 %, and produced a number of abnormalities (Tables 2 and 3). Four of the abnormal embryos lacked a head: in these the head mesoderm, which would have been invaginating at the time of treatment, had evidently been affected by the treatment more than other parts.

3. The 5 hr. treatment had more severe effects, killing 48·4 % of the 16 hr. embryos and producing abnormalities in 22·6 %. Most of the abnormal embryos were dwarfs, suggesting that both head and trunk mesoderm had failed to achieve their normal extent on invagination.

4. The 12 hr. embryos had reached the short streak (*S*) or medium streak (*M*) stage (Waddington, 1932). Only one out of eight which were treated for 3 hr. was certainly affected: this had its head region reduced. A case of duplicitas posterior arose in this group, but could not be directly attributed to the high temperature.

5. 12 hr. embryos were, however, severely affected by a 5 hr. treatment. Over 80 % were abnormal or had been killed. All the abnormalities could be correlated with the stage of gastrulation reached in individuals at 12 hr. incubation. It was

clear that each mesoderm region is most susceptible to high temperature at the moment when it is invaginating.

6. Of the 7 hr. embryos (no streak yet visible), 31 % died after a 5 hr. exposure to 45·5° C., and 25·8 % were abnormal. Since many of the controls were also abnormal, it was suggested that the 7 hr. embryos were specially susceptible to the cooling experienced when the eggs were opened for observation before treatment.

7. Unincubated embryos, in which virtually no morphogenetic movements are taking place, were unaffected by a 5 hr. treatment at 45·5° C.

8. Taken as a whole, these preliminary investigations show that in the chick as well as in Amphibia, gastrulation may be arrested by high-temperature treatment. The invaginating cells are those most affected by high temperature, possibly owing to their amoeboid nature. The results also indicate that longitudinal extension of the invaginated tissues is inhibited by high temperature.

9. Other aspects of the abnormalities have been discussed, and it is concluded that in the chick as in Amphibia, the normal differentiation of the nervous system depends upon a normal invagination of the axial mesoderm. It is desirable that further work should be carried out on the physico-chemical properties of the invaginating cells.

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EXPLANATION OF PLATE

Fig. 1. Embryo treated at 19 hr. stage: heart lying anterior to head. (×20.)

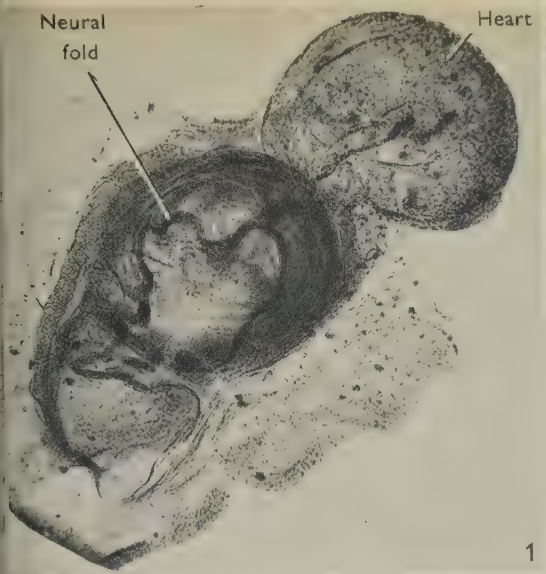
Fig. 2. Distorted, shortened embryo resulting from treatment at 19 hr. stage. (×15.)

Fig. 3. Embryo treated at 12 hr. stage: primitive streak killed, but area vasculosa well developed. (×6.)

Fig. 4. Inhibition and distortion of posterior development in embryo treated at 12 hr. stage. (×15.)

Fig. 5. Neural folds distorted by mass of uninvaginated mesoderm: embryo treated at 12 hr. stage. (×6.)

Fig. 6. Transverse section of embryo treated at 12 hr. stage, showing doubling of notochord beneath the broad neural plate.



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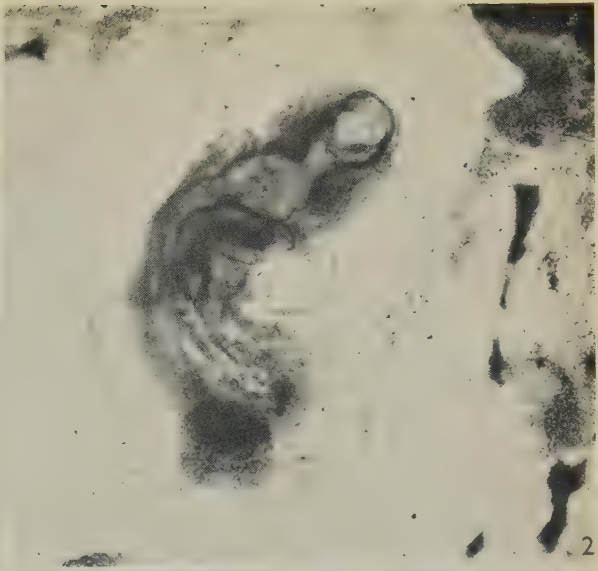

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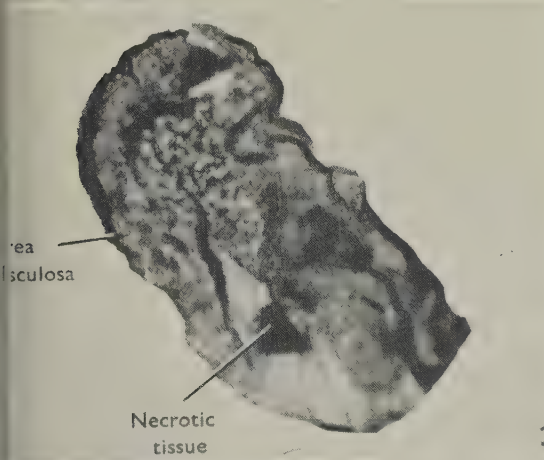

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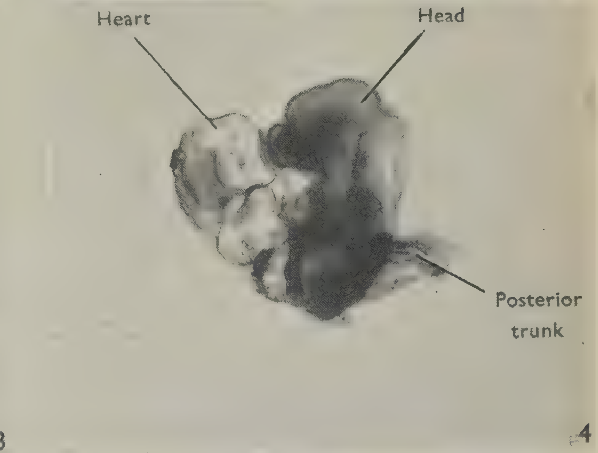

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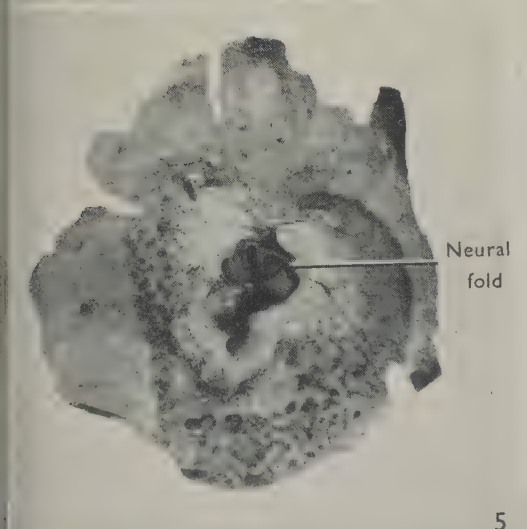

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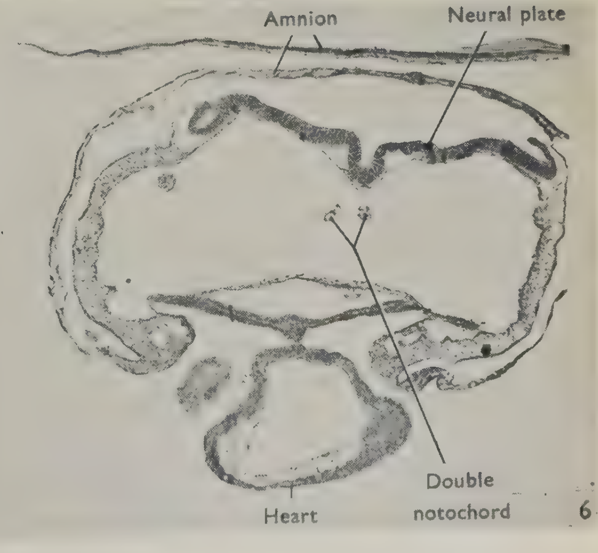

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EUCHAR—THE EFFECT OF A HIGH TEMPERATURE SHOCK ON EARLY MORPHOGENESIS IN THE CHICK EMBRYO

OBSERVATIONS ON THE ADRENAL BLOOD VESSELS IN THE RAT

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INTRODUCTION

In view of the increasing interest in the physiology of the adrenal gland, it is obviously of great importance that renewed inquiry should be made into the details of its blood supply in readily available animals. In the work to be described, a study was made of the vascular pattern of the adrenal in the rat. Satisfactory results were achieved by injection methods, employing indian ink and Neoprene Latex.

In his work on the adrenal gland of the dog, Flint (1900) described a plexus of finely branching and anastomosing arteries within the capsule, and stressed the importance of this arrangement as a means of equalizing the different pressures in the arteries of supply to the organ. He noted four types of vessel arising from this plexus:

(1) Small arteries intrinsic to the capsule (arteriae capsulae); (2) arteries to the medulla which run through the cortex without branching (arteriae medullae); (3) arteries passing into the cortex to varying depths and there ending in capillaries (arteriae corticis); and (4) a capillary system derived from the arterioles of the capsular plexus, passing centripetally in the reticulum to be continuous in the juxta-medullary part of the cortex with the first order of collecting tributaries of the central vein of the medulla. This central vein, in Flint's opinion, drained both cortex and medulla, twigs from the latter either entering it directly or joining its radicles.

Bennett & Kilham (1940), in the cat, described the superficial arterial plexus as subcapsular, not intracapsular. They considered that this plexus possessed a system of anastomosing capillaries, from which the cortical capillary network was derived. They further reported the presence of arterio-venous anastomoses on the surface of the gland. In their specimens Bennett & Kilham found neither arteriae capsulae nor arteriae corticis, but they described a number of arterial loops, both ends of which were found in the subcapsular plexus, while the curve of each loop pursued a course through the cortex to a variable depth, usually without branching *en route*. Gersh & Grollman (1941), working on the mouse and rat, also noted these arterial loops, but in addition found Flint's arteriae corticis.

Recently Harrison (1951), after ligating individual arteries of supply in the living animal, demonstrated an immediate appearance of areas of necrosis in the zona fasciculata of the rabbit's adrenal. He suggested that individual adrenal arteries are end arteries to the zona fasciculata in the rabbit and probably also in the cat and rat. It will thus be seen that there is some divergence of opinion on the details of the blood supply to the adrenal gland.

MATERIAL AND METHODS

In the present investigation, adult male albino rats were used and these were killed in each case with coal gas.

A. *Injections*

Prior to any injection procedure, whether arterial or venous, the following perfusion technique was employed: immediately after death the abdomen and thorax were opened, care being taken not to damage the diaphragm and its vessels, and a glass cannula tied in the thoracic aorta; the inferior vena cava was cannulated near its entry into the right atrium, the cannula being led to waste. The animal was then perfused via the aortic cannula at a pressure of 60–70 mm. Hg with a warmed 1·5 % solution of $NaNO_2$ in normal saline. Under nembutal anaesthesia the normal range of systolic blood pressure in the rat is stated to be between 60 and 120 mm. Hg (Griffith, Jeffers & Roberts, 1942). The duration of the perfusion varied, but blanching of the kidneys was taken to be a satisfactory index of completion.

The following injections were made:

(1) *Indian ink.* (*a*) Into the thoracic aorta in a series of five rats. (*b*) In another five rats, into the I.V.C. just above its formation, after ligating it in the thorax.

(2) *Neoprene Latex.* Injections as for indian ink in an arterial series of five rats and a venous series of five rats.

In a further series of five animals, simultaneous arterial and venous injections were made using diluted Neoprene Latex coloured with the normal red and blue pigments (B.B. Chemical Co. Ltd.). This was in an attempt to demonstrate arterio-venous anastomoses. The Latex preparations were dissected *en bloc*, with or without maceration, using a binocular microscope. In the ink preparations both adrenals were removed, embedded in celloidin and sectioned at 100μ. Some sections were lightly stained with Delafield's haematoxylin for nuclear definition. In one instance, after ink injection, the gland was dehydrated, cleared in xylol and the capsule (plus the subcapsular connective tissue) was removed by microdissection and mounted in Balsam. Another animal was perfused with 10 % formaldehyde solution for $\frac{1}{2}$ hr. followed by haematoxylin diluted 1 in 10, in order to stain the vascular endothelium; following this the adrenals were embedded in paraffin, sectioned at 8μ and lightly stained with haematoxylin and eosin.

B. *Other methods*

(1) Slices of fresh adrenal gland were stained for lipoids in Flemming's solution.

(2) A silver impregnation method (Long, 1948) was used to demonstrate the glandular reticulum.

OBSERVATIONS

Extracapsular vessels

(1) *Arteries*

After Latex arterial injection it was possible to define the arteries of supply to the gland. The arrangement was different on the two sides. On the right, a branch from the renal artery gave two twigs to the adrenal and then continued, supplying the abdominal aspect of the diaphragm; these two adrenal arteries approached the gland medially and inferiorly respectively. The left adrenal was always easier to inject than the right because of a larger number of arteries of supply:

(*a*) A superior adrenal artery arising from the aorta just below the diaphragm, sending two or more twigs to the diaphragm and ending in two terminal branches approaching the gland from above.

(*b*) Two middle adrenal arteries from a common stem arising from the ventral aspect of the aorta, and approaching the gland on its medial side. From this common stem there also arose one or more diaphragmatic branches.

(*c*) A small inferior adrenal artery from the left renal. This description is not in accord with that of Greene (1935); she described superior and inferior adrenal arteries on both sides. As was noted by Harrison (1951), the adrenal arteries of both sides broke up into a spray of finer arteries just short of the glands, and these were distributed over both anterior and posterior aspects of the glands. Anastomoses between these vessels were not observed, but small branches from them to the immediate peri-adrenal tissues were frequently found.

(2) *Veins*

There is a single adrenal vein from each gland. On the right side this drains directly into the I.V.C. and on the left it joins a diaphragmatic vein, which empties into the left renal vein. Venous Latex injections disclosed a number of small tributaries of both adrenal veins, draining the diaphragm, peri-adrenal and peri-renal tissues. Each adrenal vein was also joined by a few small venules which, having pierced the capsule of the gland, were seen on its surface. On careful dissection these last vessels were found to arise in the cortex immediately surrounding the emerging central or adrenal vein. (See later description of the central vein.)

The subcapsular plexus

As already stated, individual adrenal arteries break up into a spray of fine vessels and these, passing over the surface of the gland, pierce its capsule abruptly along their course. Then, lying in the subcapsular reticulum, each undergoes a tree-like division ultimately down to capillaries. A free arterial and arteriolar anastomosis exists between neighbouring tree systems; in spite of this there is an obvious patchiness in distribution of the arterial plexus as seen by Latex injections, some areas showing no injected vessels. When, however, the subcapsular plexus is examined in an ink-injected specimen after dissecting away the cortex from the inner surface of the capsule, a capillary network can be seen as a continuation of the arteriolar plexus (Pl. 1, fig. 1). This network is evenly distributed in the subcapsular reticulum, and from it arise the capillaries to the zona glomerulosa.

Cortical capillaries and reticulum

Using the silver impregnation method of Long (1948), the reticular tissue on the deep surface of the capsule was seen to be continuous with that of the zona glomerulosa. In the outer part of this zone, the reticulum is predominantly arranged in a honey-comb pattern surrounding small clusters of the parenchymal cells. From this honey-comb system, radial extensions of reticulum pass to the inner zona glomerulosa and continue on into the zona fasciculata (Pl. 1, fig. 2). The cells of this deep part of the zona glomerulosa are grouped in tubular fashion around the radial reticular processes. Running in this reticular framework, the cortical capillaries pass inwards from the subcapsular plexus, freely anastomose in a honey-comb pattern in the outer zona glomerulosa and continue radially through the deeper zona glomerulosa to the zona fasciculata (Pl. 1, fig. 3). Capillary anastomoses in the deeper zona glomerulosa do not appear to be so numerous as in the outer part of this zone.

In addition to the extension of these reticular processes from the zona glomerulosa, there are also long septa which run from the capsule straight through the zona glomerulosa, in some cases, as far as the zona reticularis (Pl. 1, fig. 2). The reticulum of the zona fasciculata is disposed in radial strands between the columns of parenchymal cells with numerous bifurcations and cross-connexions.

The corresponding vascular pattern for this zone shows a radial inclination of the capillaries, but with frequent departures from a simple parallel course. Bifurcation is common and cross-connexions, while present throughout the zone, are more numerous in its deeper part. Certainly the number of injected capillary cross-connexions was far less than the number of transverse reticular strands; and even allowing for inadequate ink penetration and the fact that, in a given tissue, not all the capillaries are patent at the same time, it seems unlikely that the capillary pattern of this zone rigidly follows the intricacies of its reticular support.

It has been suggested by Bennett & Kilham (1940) that in the cat the capillaries of the outer zona fasciculata may possibly be narrowed by the mechanical pressure of lipoids contained in the cells of this region. This contention is supported by the present observations on the rat. Sections treated by Flemming's method show the major distribution of lipoids to be in the outer layers of the zona fasciculata and also in the zona glomerulosa (Pl. 1, fig. 4). The transected capillaries of the outer and inner regions of the zona fasciculata after perfusion with 1:10 haematoxylin and light staining with haematoxylin and eosin are shown respectively in Pl. 1, figs. 5 and 6; both sections are taken from the same gland. Although probably dilated beyond their normal diameters by perfusion, it will be seen that the average bore of the vessels in fig. 5 is much smaller than in fig. 6.

In the zona reticularis, the capillaries are wider than those of the zona fasciculata. Here again the reticular pattern (Pl. 1, fig. 7) is a useful guide in assessing the extremely rich inter-capillary anastomosis of the region; it is arranged in a latticework so finely divided that individual cells are frequently walled off from their neighbours. Bordering on the medulla some of the capillaries become dilated and constitute the first order of collecting radicles of the adrenal vein. A smaller proportion, however, passes directly into the medullary capillary bed.

Arteries of the cortex

A study of the injected material shows that the course of the cortical arteries is very variable. They may run radially and straight to their destinations, or they may describe loops, the terminal ends of which end in capillaries which join the basic cortical capillary network. In one instance, after arterial Latex injection, the proximal stem of such a loop was found to penetrate as far as the cortico-medullary junction and there it broke up into three branches. These branches curved back through the zona reticularis and terminated in the capillary bed of the outer zona fasciculata. It was noted in this case that, while the proximal limb of the loop was easily detached from the gland parenchyma, the terminal limbs were awkward to dissect out, the reason being that they contributed small branches to the zona reticularis as they passed through it. These findings in relation to the cortical arteries in the rat supplement those of Flint (1900) in the dog. He described a few ultimate arterioles passing from the capsular plexus through the zona glomerulosa to end in capillaries in the zona fasciculata. More recently, in the mouse and rat, Gersh & Grollman (1941) have noted similar penetrating arteries and arterioles of the cortex, most of which terminate in the capillary bed of the zona fasciculata.

Bennett & Kilham (1940) and Gersh & Grollman (1941) have described another arterial form, in the cat and the rat respectively; arterial loops, both ends of which are found in the arterial capsular plexus curving to variable depths into the cortex. A similar loop is shown in Pl. 2, fig. 8. The apex of this loop reaches the outer zona fasciculata and gives a fine branch which passes deeper into this zone before returning to end in the capillary plexus of the zona glomerulosa. In Pl. 2, fig. 9, a large penetrating artery can be seen, breaking up into capillaries in the zona reticularis. Thus it seems that in the rat, at any rate, the perforating arteries to the cortex, although mainly terminating in the outer zona fasciculata and zona glomerulosa, are also distributed to the zona reticularis.

Vessels supplying the medulla

The capillary plexus of the medulla is contributed to by two sorts of vessels.

(1) Capillaries crossing the cortico-medullary junction in strands of reticulum from the zona reticularis. These vessels contain blood which having passed through the length of the cortical capillaries is presumably incompletely oxygenated and will contain hormonal products of the cortex. Only a small proportion of the capillaries of the zona reticularis is seen to pass into the medulla in this way, for by far the majority of them dilate to become the peripheral radicles of the adrenal vein.

(2) The arteriae medullae of Flint (1900) are directly derived from the subcapsular arterial plexus and pass through the cortex without branching to end in capillaries in the medulla (Pl. 2, fig. 10). These vessels are easily found after arterial Latex injection and dissection of the gland, and show striking differences in calibre within the same gland. On following them back to their origins (and here Latex preparations are valuable) some arise as large branches of a principal stem artery in the subcapsular plexus, while others derive from much smaller vessels of the plexus. The immediate terminal branches of the medullary arteries often show varicosities of a diameter exceeding the parent artery. Latex will not penetrate the subsequent capillaries but ink preparations show a mergence of these into the general capillary bed of the medulla. In any one gland, it was unusual to find more than four arteriae medullae. Bennett & Kilham report 'numerous' medullary arteries in the cat, and Gersh & Grollman describe a single artery in the mouse passing through the cortex alongside the adrenal vein. The arteriae medullae are end arteries to the medulla in that they give no branches to the cortex and except through the medullary capillaries they are not inter-connected.

Medullary capillaries

The capillaries of the medulla are arranged in a network far coarser than any seen in the cortex (Pl. 2, fig. 11). The actual number of capillaries visible by ink injection per unit area of medulla is far smaller than for any part of the cortex. The blood flow from the capillaries is to the larger collecting veins, tributaries of the central vein (see later). In the cat, Bennett & Kilham (1940) were able to demonstrate that the majority of medullary cells presented one end towards a collecting vein and the other towards a medullary capillary receiving fresh blood from a medullary artery; thus they postulated secretory and nutrient 'poles' respectively for these cells. Direct contact of cell with vessel wall was not considered an absolute criterion for the theory provided that proximity could be shown. While such an arrangement can be found in the rat, its degree of constancy is not such that it can be recorded as the normal arrangement in this animal. An obvious difficulty in comparing the two animals (cat and rat) lies in the fact that the cat possesses numerous medullary arteries each of which can be traced into capillaries, while the rat medulla receives four or less of such arteries. Silver impregnation shows the medullary cells to be grouped in clusters each around a small core of reticulum; there does not appear to be much permeation of reticulum between individual cells (Pl. 1, fig. 7). A corresponding relationship holds between medullary cells and capillaries.

The central vein

A Latex cast of the adrenal vein and its mode of formation by tributaries within the medulla is shown in Pl. 2, fig. 12. As already stated, most of the capillaries of the zona reticularis become dilated as they approach the medulla and constitute the first order of collecting vessels. These, in turn, drain into second-order collecting veins which run parallel to the capsule at the junction of cortex and medulla (Pl. 2, fig. 11). By a confluence of two or more of these a third-order vessel is formed which inclines radially into the medulla. Further confluence results in fourth- and, often, fifth-order veins before the stem of the central vein is reached. The medullary capillary plexus drains largely into the third- and fourth-order vessels. As the central vein pierces the cortex to emerge from the gland it is joined by a series of small veins formed in the zona reticularis by a confluence of capillaries. Only in the cortex in the neighbourhood of the central vein were such cortical veins demonstrated. Latex injections indicate that some of these cortical veins run in a centrifugal direction and join the main vein immediately under the capsule or just external to it, but they do not, in the rat, constitute a subcapsular venous plexus, as described by Flint (1900) in the dog.

DISCUSSION

It will be seen from the above description that the blood vessels in the rat adrenal are so arranged that they will allow of a centripetal circulation. Further, the presence of penetrating arteries may permit rapid adjustments in the circulatory condition throughout the gland. Such vascular arrangements deserve some further attention from the point of view of their possibly being concerned in the adjustments in the gland in response to functional requirements.

Yoffey & Baxter (1949) have indicated by histochemical methods that lipoid production in the adrenal cortex of rats is mainly from the zona fasciculata, and suggest that the zona reticularis is principally a storage depot for such bodies. Furthermore, if the animals, prior to killing, were injected with adrenocorticotropic hormone a resulting depletion of lipoid occurred in the zona reticularis with a greater concentration in the zona fasciculata. It is apparent from a study of Pl. 1, figs. 4–6, that where the intercellular lipoid is in highest concentration the neighbouring capillary lumina are smallest; this is possibly, as has been discussed above, due to compression of the vessels by fat-laden cells. It is significant that most observers have noted that the arteriae corticis usually spend themselves in the capillary bed of the zona fasciculata and reticularis. In the rat, extreme inconstancy was noted in the demonstration of arteriae corticis and cortical arterial loops in what otherwise appeared to be satisfactory ink penetrations. Possibly the degree of stress to which the animal is subjected in the process of capture and killing is an important factor. Such cortical arteries may play an important part in rapidly increasing or decreasing the blood supply to the zona fasciculata and reticularis under different physiological conditions. An increased blood flow to these zones could remove larger quantities of cortical hormones into the blood stream. Selye (1950) has postulated an increased secretion of cortical hormones under conditions of stress as a result of a larger output of ACTH. As an integral part of the Stress Adaptation Syndrome of Selye there is also an increased production of adrenaline by the adrenal medulla resulting from autonomic nervous stimulation.

The patency of, and blood flux through, the basic capillary bed of the gland are obviously an important controlling factor on which the systemic blood titre of cortical hormones depends. It would therefore be of great interest to assess the effect of controlled stress to the animal on the intimate vascular pattern of the adrenal glands, particularly in relation to the demonstrable number and size of the arteriae corticis and arteriae medullae. Gersh & Grollman (1941) found that after prolonged stimulation by thyroid dosage in mice, the adrenal glands showed cortical hypertrophy with an increase in capillary number and diameter; they make no conclusive statement as to the effects of short-lived stimulation beyond stating that in such cases the capillaries can be more completely injected than in the normal gland.

Since arteriae medullae and arteriae corticis have a common field of origin in the subcapsular arterial plexus, it may be that under conditions of physiological stress an increased flow occurs along both these routes with a simultaneously increased lavage of both cortical hormones and adrenaline into the general circulation.

SUMMARY

A macroscopical and microscopical study of the adrenal blood vessels was made in the rat after indian ink and Neoprene Latex injections.

1. The circulation is centripetal; from a subcapsular arterial plexus capillaries run through the cortex in the glandular reticulum and the majority empty into radicles of the central vein of the medulla.
2. A small proportion of the cortical capillaries continues over the corticomedullary junction into the medullary capillary bed; this also receives the arteriae medullae, end arteries derived from subcapsular plexus.
3. Penetrating arteries from the subcapsular plexus terminate in the basic capillary bed of the cortex mostly in the zonae fasciculata and reticularis.

The suggestion is made that these arterial forms, additional to the basic capillary pattern, may under different physiological conditions provide a means of rapidly altering the vascular flux through both cortex and medulla.

I wish to thank Prof. J. D. Boyd for his guidance and criticism in the preparation of this paper. I gratefully acknowledge technical advice from Mr J. Cash, and I am greatly indebted to Mr J. A. F. Fozzard for the photographs.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. An indian ink injection of a representative field of the subcapsular plexus, adherent to the deep surface of the adrenal capsule, in the rat. The cortex has been dissected away piecemeal and the specimen cleared and mounted. A fine artery is seen branching right down to a capillary plexus. × 60.

Fig. 2. From a silver impregnation of rat's adrenal. The reticular framework of the zona glomerulosa and outer zona fasciculata is demonstrated. Note the long strand passing radially inwards from the capsule and the tubular arrangement of cells on the inner zona glomerulosa around short radial strands of reticulum. 8 μ. × 500.

Fig. 3. From cortex of an indian ink injection of rat's adrenal. Note honey-comb pattern of capillaries in the outer zona glomerulosa and the radially directed capillaries of the inner zona glomerulosa and the zona fasciculata, with cross-connexions. Compare with fig. 2. × 500.

Fig. 4. A segment of rat's adrenal cortex stained with Flemming's solution to show lipoid distribution. This is maximal in the zona glomerulosa and outer zona fasciculata. Note the transitional zone between the two, devoid of lipoid. 8 μ. × 168.

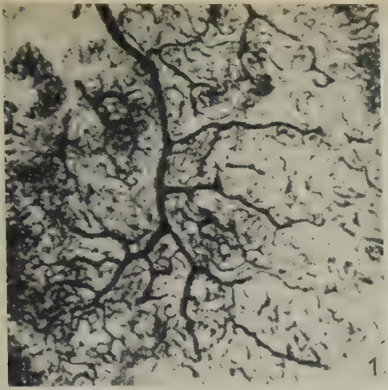
Figs. 5 and 6. Transected capillaries in the outer and inner regions of the zona fasciculata respectively. Taken from the same gland after perfusion with haematoxylin and subsequent cellular staining with haematoxylin and eosin. Note the endothelial linings with occasional nuclei. Further, note the average capillary diameter in fig. 5 is less than in fig. 6. Correlate this observation with the distribution of intracellular lipoid in fig. 4. 10 μ. × 280.

Fig. 7. From a silver impregnation of rat's adrenal, showing the reticular pattern of the medulla and the transition to the zona reticularis. Note the almost acinar-like arrangement of medullary cells around a core of reticulum. Note the very fine division of the reticulum in the zona reticularis. The irregular space to the left is a collecting vein. Light haematoxylin nuclear stain. 8 μ. × 500.

Plate 2

Fig. 8. From an indian ink injection of rat's adrenal, showing part of the two main limbs of a small arterial loop, the base of which reaches the outer zona fasciculata. From the right-hand limb a fine vessel passes into the capillary bed of the zona glomerulosa. Another fine branch runs a curved course from the base of the loop, and also joins the capillary bed of the zona glomerulosa. 100 μ. × 262.

Fig. 9. A cortical artery terminating in the capillary bed of the zona reticularis. Well-filled radicles of the adrenal vein are visible in the medulla. Indian ink injection. 100 μ. × 112.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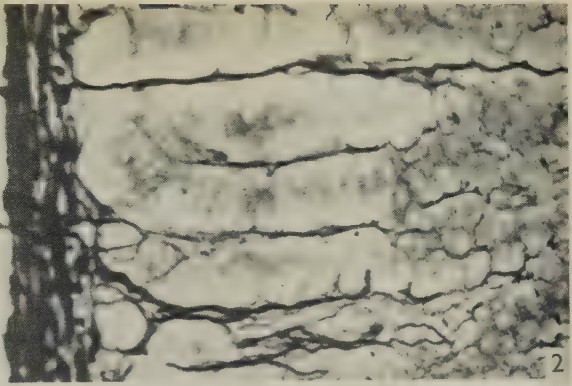
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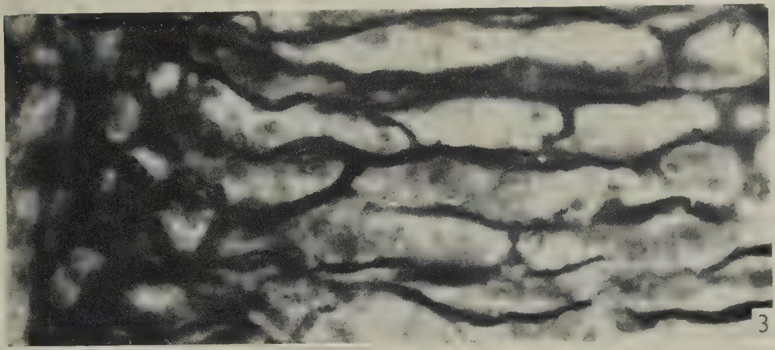
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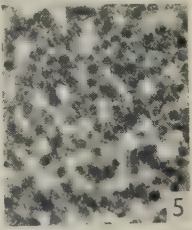
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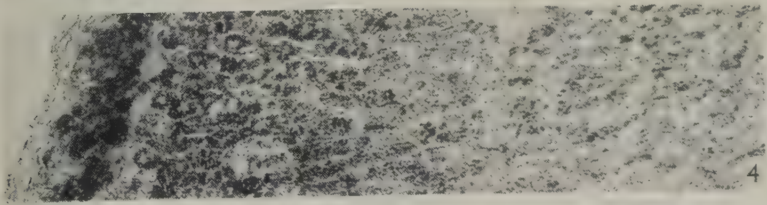
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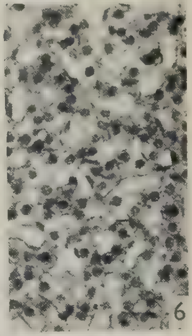
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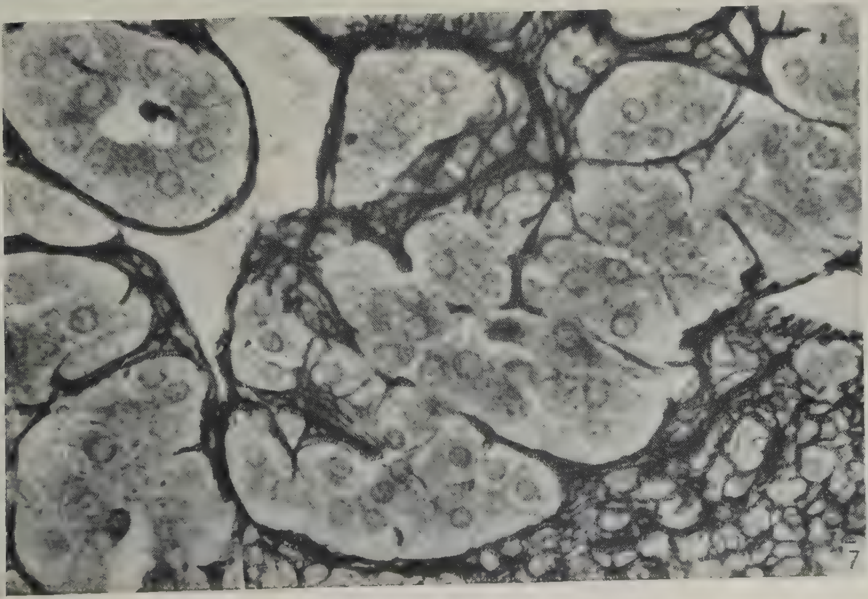
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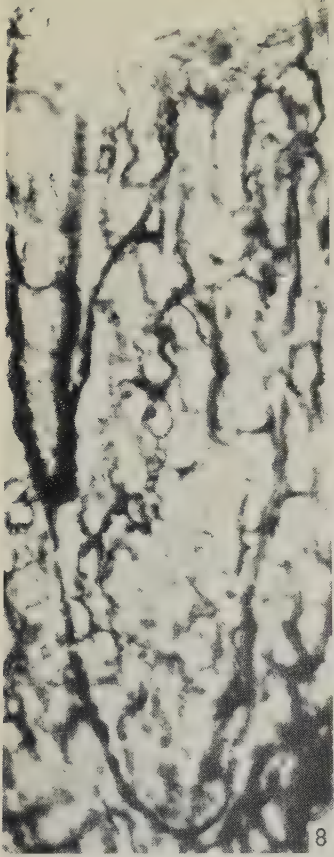
LEVER—OBSERVATIONS ON THE ADRENAL BLOOD VESSELS IN THE RAT



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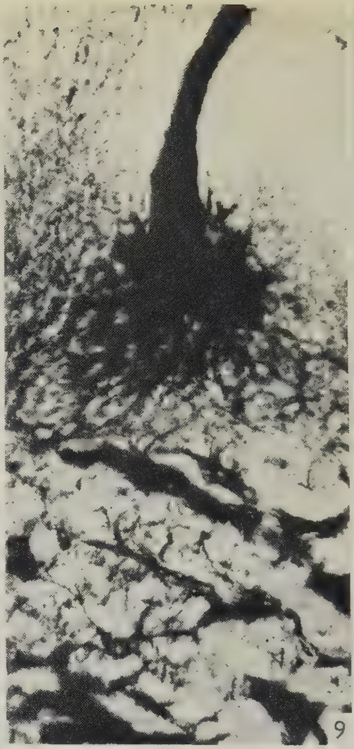

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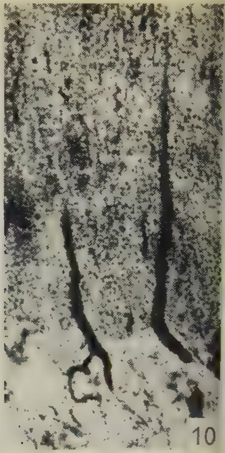

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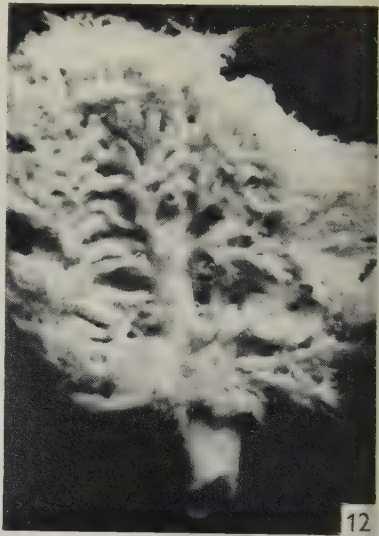

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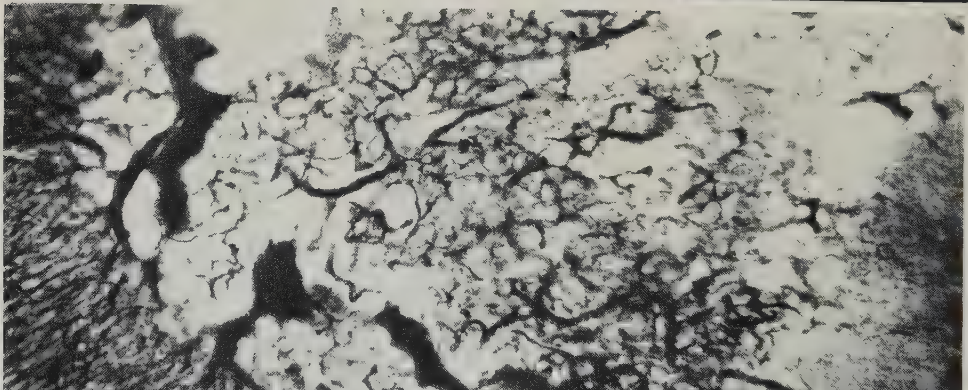

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LEVER—Observations on the adrenal blood vessels in the rat

Fig. 10. Two arteriae medullae in an ink injection of rat's adrenal. The termination in the medullary capillary bed of one of the arteries is seen. 100μ. ×112.

Fig. 11. From an ink injection of rat's adrenal, showing the dense pattern of the capillary plexus in the zona reticularis and the dilation of some of these capillaries at the cortico-medullary junction to form the first order of collecting veins of the central vein showing as a space, top left of figure. Second-, third- and fourth-order venous tributaries can be seen. Note the medullary artery, bottom right, and the capillary plexus of the medulla. Some of these capillaries can be seen running into the larger collecting veins. 100μ. ×112.

Fig. 12. A Latex cast of the adrenal vein and its tributaries in the rat. ×18.

REVIEWS

Die Rolle der einzelnen Labyrinthrezeptoren bei der Orientation von Augen und Kopf im Raume. By J. Szentágothai. (Pp. 129; 48 figures.; $6\frac{1}{2}\times 9\frac{1}{2}$ in.) Budapest: Akadémiai Kiado. 1952.

This short monograph, which is dedicated to the memory of Högyes, is concerned with the activity of the reflex pathways connecting the labyrinth and the extra-ocular muscles. It summarizes the extensive work of the author and his co-workers in the University of Pécs on the anatomical connexions between the labryinthine receptors and the oculo-motor nuclei and includes an account by Gömöry of the reflex connexions between the cristae acusticae and the neck muscles. Beautiful techniques are described for the stimulation, by micro-cannulae, of different parts of the labyrinth and for the recording of activity in the muscles under consideration. The advantages of direct stimulation of the labyrinth in enabling objective records to be made are stressed, but the technical difficulties and the problem of controlling the experimental conditions are frankly admitted. As a result of his studies the author considers that he has succeeded in demonstrating the effectiveness of ampullopetal flow in the horizontal semicircular canal and of ampullofugal flow in the vertical canals in the production of eye, ear and neck-muscle reflexes. Direct proof of reflexes arising by displacement of the membranous ampullae, the necessity for which was first inferred by Lorente de Nó was also found. The fundamental properties of these reflexes are described, and it is suggested that they probably have a dominating role in linear acceleration and act as positional receptors (*schnelle Lagerezeptoren*) or retarded movement receptors (*verlangsamte Bewegungsrezeptoren*) in assuring the transition between endolymphatic flow reflexes and macular reflexes.

From both maculae it was found possible to obtain symmetrical and asymmetrical ocular reflexes. To explain this finding a functional localization of the different muscles on the macular surface is assumed and discussed in detail for the representation of the eye muscles in the macula of the utriculus.

On the mechanism of connexion between the labyrinth receptors and the effector organs the author has much of significance to report. This is particularly so with regard to those of the semicircular canals, where it is believed that each canal has a direct and anatomically localized connexion with two ocular muscles related in activity to movement in its own plane. This connexion is by way of a reflex arc consisting of only three neurones, the intermediate one being in the posterior longitudinal bundle. The arc is characterized by a high degree of differentiation and specialization and by a high speed efficiency. In addition to this system there is a much more complicated one consisting of shorter intercalary neurones in the substantia reticularis. It is believed that this 'slower' system is largely responsible for the reciprocal inhibition of antagonists and for the selection of muscles which are synergistic in different combinations. It is suggested that this intercalary system possesses a considerable adaptability in respect to changed anatomical and functional conditions. Thus it vicariously takes over the mediation of the specific reflexes within a few days of destruction of the longitudinal bundle, though the reflexes are considerably slowed down.

Having regard to the importance and difficulties of the problems investigated, the text is very short, almost terse, and the anatomical control of the interpretations of the experimental findings is not always satisfactory. In particular, the description of the localization of muscles in the oculomotor nuclei is not completely convincing. Nevertheless, Prof. Szentágothai has made available data of considerable importance and his interpretations are striking in their reasonableness. In more than one direction his contribution is an impressive one to our knowledge of the part played by the internal ear in the reflex control of muscular activity.

J. D. BOYD

1st Annual Report on Stress. By HANS SELYE. (Pp. xxxi+511.) Montreal: Acta Inc. 1951.

If the word 'stress' and the concept of the 'adaptation syndrome' do not become established in the common parlance of medicine, the fault will not be Dr Selye's. After repeated elaborations of his idea in journals, as well as in his text-book on Endocrinology, he published in 1950 a bulky volume entitled *Stress*, which he described as 'a treatise based on the concepts of the general-adaptation-syndrome and the diseases of adaptation'. The work which is the subject of the present review is a follow-up, and judging by its title it is likely to be the precursor of a series which Dr Selye proposes to issue annually.

It is none too easy to write a review of a book of this kind. The adaptation syndrome, as most students of endocrinology now know, is Dr Selye's description of a series of reactions which occur when the body is subjected to such non-specific 'stresses' as exposure to extreme cold or trauma. Simply paraphrased, Dr Selye's idea is that when what he calls a 'stressor agent' operates, cortical hormone is immediately liberated by the adrenal cortex, which becomes temporarily depleted of its secretion. This is the alarm phase of the syndrome. A 'shift' in pituitary function then occurs, and production of all trophic hormones except ACTH diminishes. During this phase of so-called resistance, ACTH is poured into the blood-stream and stimulates the adrenal cortex to produce more cortical hormone. If the 'alarming stimuli' continue, the reaction breaks down and a phase of 'exhaustion' supervenes, terminating in death. 'Derailments' of this 'adaptive mechanism' are regarded as the principal factor in the production of a wide variety of diseases.

Dr Selye's generalization undoubtedly helps to systematize a number of diverse observations about the reactions of the body in a variety of circumstances. But ideas can be destroyed by over-loading, and there can be equally little doubt that the generalization helps very little, if at all, in the understanding of a high proportion of the many facts which Dr Selye pulls into his net. Indeed, it is difficult to avoid the impression that, if pressed, Dr Selye would be able to find a way to include within his general scheme almost any biological observation about the 'total' response of the body, or about the behaviour of the body in disease. He makes hardly any secret at all of his desire to make the adaptation syndrome 'a unified concept of disease', although he seems to recognize that it is nevertheless vague and that it fails to explain 'the ultimate causes of disease'. Quite apart from any question of the value of pulling into his framework many facts which appear little enriched either in shape or substance by their new environment, it is not easy to appreciate why 'derailments' of the 'pituitary-adrenal axis'—to use Dr Selye's terminology—are any more a unifying concept than 'derailments' of other bodily mechanisms. The so-called diseases of adaptation are surely associated with basic anomalies of physiological function other than the one which is Dr Selye's over-riding preoccupation—and *post hoc propter hoc* is hardly a safe switch for illuminating some blinding white truth which will suddenly lend understanding to all and sundry facts of medicine.

Dr Selye is thoroughly aware of the critical attitude which his writings have provoked in a very large number of physiologists. He opens his new book by reviewing the reviews accorded his previous volume. In a section headed 'General Approvals', he quotes anonymous writers who commend his work as a landmark in the history of medicine, and as the work of genius. In another, headed 'General Disapprovals', he refers—to cite only one example—to a critic who deplores the extravagance of his concepts. If only the concepts were plural and extravagant. Dr Selye's concept is, in fact, so singular and so simple that one can turn over the leaves of his book, forgetting concepts entirely, and treat the volume as a not too critical review of endocrinological and clinical literature—attractively coloured by its author's evangelistic attitude to his pet idea, and in places by very odd writing. One can easily forgive Dr Selye his striving and perhaps also his literary shortcomings—for he makes no secret of the fact that the book is a transcription of something that was poured into a dictaphone. But it is difficult to overlook what seems to be a sparing use of his more critical abilities. He gives the impression that one scientific paper is as good as the next, provided the conclusions it reports can be made to fit within the framework of the adaptation syndrome. If, instead of taking pride in the fact that in 1950 he had been able to compile 3000 references 'pertinent' to his subject, as compared with a tenth as many in 1937, he had indicated which of them related to experimental work that had been precisely controlled, or to clinical observations that had been carefully checked, he would have served students of endocrinology far better than he has done. For while he exhorts his critics to take the necessary time 'to examine the validity of each fact and reasoning' upon which his 'entire stress concept' is based, he gives little indication that he himself has considered how many of the 3000 studies he quotes in fact test the validity of his thesis, and how many are merely clinical or other reports which incorporate the words stress, adaptation, or alarm without in any way adding to the information they convey.

S. ZUCKERMAN

The 'Neurility' of the Kidney. By C. G. De Muylder. (Pp. xiv + 80; 44 illustrations; $10\frac{1}{2} \times 8$ in.; 25*s*. net.) Oxford: Blackwell Scientific Publications. 1952.

Some fourteen years ago Dr De Muylder began an investigation of the nerve supply of the kidney. As his research progressed it soon became apparent to him that the whole problem of renal innervation, especially from the pathological aspect, required to be investigated. In the present book the author not only gives a detailed account of his personal contributions to the study of the neuro-vascular architecture of the kidney but also integrates much that has been done by his Belgian colleagues.

The book is subdivided into five main sections: (1) normal innervation of the kidney; (2) normal juxtaglomerular apparatus; (3) comparative anatomy; (4) possible relationship between nervous elements and some aspects of renal pathology; and (5) significance of the nervous constituent of the kidney.

Dr De Muylder describes two sets of nerves to the kidney—one by the ureteral vessels and the other by vessels that supply the part of the kidney derived from the metanephric blastema. Some of the latter nerves end in the venous endothelium, and the author puts forward the hypothesis that these nerves may be 'presso-receptors'. In the later chapters he discusses at length the rôle these nerves may have in 'crush-syndrome' and other conditions.

The book is a stimulating contribution on the rôle of the nervous system in kidney physiology and pathology. The frequent use of the personal pronoun 'we' becomes at times most irritating and monotonous. If a new edition of this work is required, it is to be hoped that this will be altered.

W. J. HAMILTON

Antibodies and Embryos. By W. F. Rogers Brambell, W. A. Hemmings and M. Henderson. (Pp. 103; 10 figures and 15 tables; $8\frac{1}{2} \times 5\frac{1}{2}$ in.; 12*s*. 6*d*. net.) University of London: The Athlone Press. 1951.

In the present publication Prof. Brambell and his colleagues have collected together and summarized the recent investigations on the passive transfer of immunity from mother to foetus or newborn young in various mammals.

The substance of the book consists of a series of lectures given by Prof. Brambell in the University of London in October 1950. The authors have been especially concerned with the following problems: (1) the passage of antigens, whether of foetal or microbiotic origin, from the uterine lumen into the maternal system; (2) the conditions of entry of maternal antibodies into the uterine lumen; (3) the transfer of antibodies and other proteins from the mother to the foetus and their utilization by the foetal tissues; and (4) the factors which permit and limit the invasion of the maternal tissues by the foetal trophoblast and of the foetal tissues by the maternal phagocytes.

The techniques used to elucidate these problems have involved the combined efforts of immunologists, embryologists and physiologists.

Until recently it has generally been assumed that the passive transfer of immune bodies in many species took place through the allanto-chorionic placenta and the freedom with which this transfer occurred in the main depended upon the structure of the placental membrane—the haemochorial placenta being most permeable, the epitheliochorial being least permeable or even impermeable. The transfer of immune bodies occurs in domestic ungulates in the colostrum.

Brambell and his colleagues have shown that in the rabbit considerable quantities of maternal plasma proteins pass through the bilaminar omphalopleure into the yolk sac on the 7th to the 8th day. They have also been able to show that agglutinins pass directly into the uterine lumen and are then absorbed by the yolk-sac splanchnopleure. If the vitelline circulation is interrupted, there is a reduction in the amount of agglutinins transferred.

The authors rightly point out that while the transfer of immunity may occur as they describe it in the rabbit and possibly in many rodents, it cannot hold for the human foetus where the arrangement of the foetal membranes is different. In the human subject the transfer of immunity must be through the allanto-chorionic placenta.

The book is most stimulating and should be consulted by those who are interested in placental permeability. It concludes with a useful bibliography. W. J. HAMILTON.

BOOKS RECEIVED

The Tissues of the Body. An Introduction to the Study of Anatomy. By W. E. Le Gros Clark, 3rd edition. (Pp. xi + 407; 124 figures; 9½ × 6 in.; 30*s*.). Oxford University Press.

Anatomie Médico-Chirurgicale. Fasc. X. La Cavité Abdominale (2nd part). By P. Bellocq. (Pp. 144; 55 figures; 8½ × 10½ in.; 1500 Fr.) Paris: Masson et Cie.

PROCEEDINGS OF THE ANATOMICAL SOCIETY

DECEMBER 1951

The Annual General Meeting of the Society for the Session 1951–2 was held in the Department of Anatomy, University College, Gower Street, London, W.C. 1., on Friday, 7 December 1951. The President (Prof. W. E. Le Gros Clark) was in the Chair.

The following are the authors' abstracts of the papers presented.

The measurement of lactating mammary epithelium. By K. C. Richardson. *University College, London*

Recent experiments on the artificial induction of lactation in ovariectomized virgin goats by the administration of steroid hormones have shown the need for some form of measurement of the glandular tissue in addition to the usual qualitative description of growth abnormalities as seen in sample sections (Cowie, Folley, Malpress & Richardson, 1951, *J. Endocrinol.* 7). Some previous workers have estimated mean alveolar diameters on the assumption that the mammary alveolus is approximately spherical, but this measurement has not been correlated with milk yield or with the size of the total gland. Owing to the great variability of alveolar development in different regions of some experimental glands a large number of fields must be measured which is both tedious and time consuming. A method for the measurement of lung alveoli described by Short (1950, *Philos. Trans.* B, 235) has been applied to a series of twenty goat udders brought into lactation by treatment with oestrogen alone, or in combination with progesterone. By this new method the surface area of the total secretory epithelium in a half udder can be calculated, and the results show a high degree of correlation with the total milk yields for each gland over selected time intervals.

The development of the so-called 'uterus masculinus' in the rabbit. By E. B. Carr. *University College, London.* (Introduced by M. Abercrombie)

Its position and anteriorly bilobed structure have led to the conclusion that the 'uterus masculinus' of the rabbit is the male homologue of the female uterus and therefore Müllerian in origin. A study of the development of this organ shows that it begins to form at 23 days by the swelling and lobulation of the fused bases of the Wolffian canals, thus forming a Wolffian sac. The Müllerian tissue is at this stage a remnant on the ventral wall of this sac. The Wolffian sac subsequently enlarges considerably, while the openings into it of the vasa deferentia (formed from the upper ends of the Wolffian canals) migrate posteriorly so that at 32 days they open at the base of the sac. By this time the remnants of the Müllerian canals appear as only a few cells embedded in the ventral wall. The 'uterus masculinus' therefore develops from Wolffian tissue, and it is suggested that the term 'vesicula seminalis' should be used, there being no homologue in the female rabbit.

Factors affecting speed of movement of fibroblasts in tissue cultures. By J. E. M. Heaysman. *University College, London.* (Introduced by M. Abercrombie)

An analysis of the factors affecting the movement of cells in tissue culture may throw light on the control of cell movement in the whole organism. With this in view individual variations in velocity of chick heart fibroblasts within each of a number of liquid hanging drop cultures have been investigated. The velocity of an individual cell is negatively correlated with the number of other cells with which it is in contact; but where the number of contacts is constant, it is not apparently related to the density of population of surrounding cells. There is also a transitory acceleration produced by losing or gaining a contact with another cell. When a cell has large numbers of contacts its velocity is also affected by the velocity of its neighbours. These phenomena may be explained in terms of a tendency of the cells to adhere to each other, and a tendency of the cells to avoid running over each other's surfaces. When allowance is made for these various effects, there still remains much variance in velocity within a culture, though none of it has been shown to be due to concentration gradients of diffusable substances.

Chromidial studies on the superior cervical ganglion of the rabbit.

By E. P. Samuel. *University of Manchester*

The presence of caudally projected postganglionic axones from cells in the superior cervical ganglion of the rabbit and of intraganglionic commissural neurones has been re-investigated. The method employed was to study the chromatolytic changes in the ganglion produced by preganglionic (twelve rabbits) or postganglionic sympathectomy (twelve rabbits). A study of normal ganglia fixed in equal parts of 10 % formalin and 95 % alcohol and stained with cresyl violet showed such an extensive range of content and arrangement of the chromidial bodies in the neurones that any formal classification of the neurones on this basis was impossible. The ganglia from animals subjected to preganglionic sympathectomy exhibited the same cellular variations, but there was no increase in the number of chromatin-free cells. It was concluded that caudally projected postganglionic axones did not exist in the rabbit. On the other hand, post-ganglionic sympathectomy caused diminution or alteration of the chromidial bodies in 98 % of the cells in the ganglia. After consideration of the ratio of affected and unaffected cells persisting after post-ganglionic sympathectomy, it was concluded that it was unlikely that intraganglionic commissural neurones were present in the superior cervical ganglion of the rabbit.

The prenatal development of the abdominal para-aortic bodies in man.

By R. E. Coupland. *University of Leeds*

The observations presented in this communication form the preliminary stage in the investigation of the development, fate and function of the abdominal para-aortic bodies of the human foetus.

Twenty-two human foetuses between the 8 mm. stage and full term have been examined, seventeen by methods of serial section and five by gross dissection. Fixatives employed include Bouin, formol-dichromate and Zenker.

In the 8 mm. embryo the sympathetic anlage forms a chain on the dorso-lateral aspect of the abdominal aorta; there is no evidence of a pre-aortic plexus. By the 12 mm. stage the anlage has extended into the pre-aortic area. At 14 mm. the invasion of the medial aspect of the epithelial adrenal by sympathetic elements has begun; there is as yet no evidence of further differentiation of the sympathetic cells.

Differentiation of the anlage into chromaffin cells is first observed in a 16 mm. embryo and is shown by the appearance of whorls of cells in the pre-aortic region between the origins of the coeliac and inferior mesenteric arteries. The first sign of cellular change is a reduction in intensity of nuclear staining.

These changes are more marked in a 19·5 mm. embryo, and a reconstruction shows collections of these extending in a cranio-caudal direction from the upper end of the abdominal aorta to the pelvis, and laterally as far as the urogenital organs. Mitotic figures are frequently observed in the peripheral zone of the whorls.

The sympathetic anlage is not fully differentiated prior to birth and primitive cells are found in the interior of the adrenal, in the sympathetic plexuses and in the para-aortic bodies up to this time. These provide a source of chromaffin cells in both the para-aortic bodies and the adrenal up to birth.

The extra-adrenal chromaffin elements are in the main arranged into compact, encapsulated bodies with a well-developed vascular system. Cells are arranged in cords between sinusoidal capillaries and are characterized by an ovoid or rounded nucleus of approximately $6{\cdot}4\mu$ diameter in which the chromatin granules are irregularly scattered giving a 'dusty' appearance. A positive chromaffin reaction was first observed in a 55 mm. foetus and appears to increase in intensity with age. In addition to these obvious bodies, non-encapsulated cells are also found—singly or in small groups—in the sympathectic ganglia.

It has been observed that in foetuses between 19·5 and 52 mm. chromaffin cells are extending into the medial aspect of the adrenal gland from closely apposed bodies. Hence it would appear that the ectodermal elements of the adrenal enter that structure in two phases: (1) the primitive sympathetic anlage at 14 mm.; (2) fully differentiated chromaffin cells at a later stage.

There is no evidence of any marked depletion of chromaffin material in any one region of the foetus prior to birth and the total amount of material increases steadily up to that time.

An apparent invasion of para-aortic bodies by lymphoid tissues has been noted in a 108 mm. foetus and larger specimens. This invasion becomes more widespread as age advances.

The optical determination of cell mass. By R. BARER. *University of Oxford*

The determination of the weight of single living cells or of cellular constituents is a matter of very considerable difficulty. Since these weights are of the order 10^{-12} of a gram, ordinary weighing methods cannot be used. A method based on X-ray absorption measurements has been developed by Engström & Lindström (1950, *Biochim. biophys. Acta*, 4), but this can only be used on dried, i.e. non-living, specimens. Further disadvantages are that the resolving power is low, the thickness of the specimen must be accurately determined and very expensive and elaborate apparatus is necessary. The development of interference microscopy, the principles of which will be briefly discussed, makes possible a new approach to the problem. This method makes it possible to measure the phase change, i.e. the product of refractive index and thickness produced by the object. This quantity can be related to the dry weight of protein in the cell by the formula

$$\text{Weight of protein} = \text{phase change} \times \frac{\text{projected area of cell}}{100\alpha}.$$

It will be observed that the thickness of the cell, which is extremely difficult to determine, does not appear in this formula. α is a constant depending on the chemical nature of the object. Reasons will be given for selecting $\alpha = 0{\cdot}0019$ for most cells. This method was used for determining the weight of protein in a single human red blood corpuscle. A mean value of $31{\cdot}4 \times 10^{-12}$ g. was found. The mean corpuscular haemoglobin can also be determined by other methods and is usually stated to lie in the range of $27\text{–}32 \times 10^{-12}$ g.

Phase-contrast microscopy of the vitreous body in cattle. By A. PIRIE, B. A. BEMBRIDGE and G. N. C. CRAWFORD. *University of Oxford*

Small pieces of the vitreous body taken from freshly removed eyes of cattle have been examined without further treatment by phase-contrast microscopy. The structures which can be seen include: (1) a thin hyaline membrane surrounding and contiguous with the underlying vitreous substance; (2) fibres $0{\cdot}5\text{–}3\,\mu$ in width within the substance of the zonular region of the vitreous and apparently forming a connexion between the latter and the ciliary processes; and (3) very fine fibrillae throughout the vitreous body.

The effect of crystalline trypsin and purified collagenase on these structures was examined with the following results: (1) the hyaline membrane was digested by both trypsin and collagenase; (2) the fibres from the zonular region of the vitreous were digested by trypsin and not collagenase; and (3) the fine fibrillae were digested only by collagenase. The significance of these results in the light of previous findings will be discussed.

A preliminary comparison between the structures visible in the vitreous body of cattle and those of the monkey and pigeon has also been made.

A study of bone growth in the rat using radioactive calcium. By D. H. Tomlin, K. M. Henry and S. K. Kon. *University of Reading.* (Introduced by W. E. Le Gros Clark)

The technique of autoradiography, with the radioactive isotope ^{45}Ca was applied to study the manner in which growing rats incorporate dietary calcium into the structure of the long bones. The rats were given a stock diet to which calcium carbonate containing this isotope was added during specific periods.

In one experiment a group of rats received, during alternate 3-month periods, a radioactive and a non-radioactive diet, and subgroups were killed at the end of each period. In the second experiment, the radioactive diet was given continuously, and subgroups were killed at intervals of 2 weeks. Both experiments began when the animals were 28 days old.

The femora and humeri were removed, and after being embedded in wax were split longitudinally, and the exposed surfaces ground flat. These surfaces were then placed in contact with 'Crystallex' X-ray film, and after exposures of a few days satsifactory autoradiographs were developed, showing the distribution of the radioactive calcium with a resolution of approximately 50μ.

When correlated with dimensional measurements, the autoradiographs showed details of the remodelling processes involved in growth. These took place with the maximum economy of bone already laid down. Resorption occurred only where existing bone material could not be incorporated into the future structure, and this was particularly striking in the early development of the diaphysis of the femur. Bone laid down in the anterior wall at the age of 28 days still remained essentially unchanged in distribution in a bone 500 days old, whereas extensive resorption completely changed the initial structure of the posterior wall. This has been interpreted as resulting from the marked curvature of the posterior wall in comparison with the straightness of the anterior wall, which allowed the latter to remain intact throughout growth.

Glycogen in human skin autografts. By A. W. Scothorne and R. J. Scothorne. *University of Glasgow*

The epidermal glycogen content of pinch autografts of human skin has been studied histochemically in a series of biopsies removed at intervals following grafting. With the method used (alcohol-formalin-picric acid fixation, Bauer-Feulgen stain), normal epidermis shows only very small quantities of glycogen. In biopsies removed at 5, 9, 10 and 12 days after grafting, glycogen is abundant in the superficial half or more of the stratum spinosum. At 20 days the epidermal glycogen is reduced to almost normal amount. The possible significance of these changes is discussed.

Comparative anatomy of the subepithelial lymphatic glands. By Kenelm H. Digby and J. B. Enticknap. *Royal College of Surgeons, London.* (Introduced by F. Goldby)

As evidence accumulates, it becomes more obvious that subepithelial lymphatic glands act as antivaccinating organs. Their cells wander to the surface, encounter bacteria and return with some of these into the lymph nodes where the bacteria are destroyed with the production of antibodies thus leading to some degree of immunity. In view of their important function a survey of their comparative anatomy is desirable.

The organs are often inconspicuous, and attempts have been made to render them more obvious. Generally they are found where bacterial activity is greatest; they tend to develop in culs-de-sac where the cells which reach the surface are less likely to be swept away and lost to the body, and afferent lymphatics are absent.

In mammals the following may be listed:

(*a*) Along the alimentary canal: faucial tonsils; guttural pouches, antral nodules or patches; duodenal, jejunal and ileal nodules; Peyers' patches; terminal ileal tonsil; proximal caecal patch, distal caecal patch, tonsil or appendix; colonic nodules and patches and rectal nodules.

(*b*) In the respiratory system: nasopharyngeal tonsils; nodules in pharynx and trachea; bronchial tonsil and bronchiolar nodules.

(*c*) In the eyelids: subconjunctival nodules and patches.

In birds which do not masticate their food, which lack a colon to inspissate the faeces and which mingle faeces and urine in a common cloaca, arrangements are correspondingly different. There are no faucial tonsils, but much oesophageal lymph tissue, bilateral ileo-rectal caeca partly or wholly lined by lymphoid tissue and a clocal bursa also wholly or in part occupied by lymphoid tissue.

In fishes subepithelial lymphatic tissue may be found about the gills, and more abundantly on the spiral valve of the dogfish and of cyclostomes.

Dimensions at the nodes of Ranvier. By J. Z. YOUNG. *University College, London*

The nodal membrane is a cylindrical portion of the axon surface not covered by myelin. It is encased in a cylinder of material continuous with the neurilemma (inner endoneurium). Outside this cylinder is a tissue space, the perinodal space, bounded externally by the sheath of Key and Retzius (outer endoneurium). Measurements have been made on photographs of mammalian fibres lightly fixed in osmium tetroxide and carefully teased in 33 % glycerin. The gap in the myelin is near the limit of resolution with white light and ranges from $<0{\cdot}5\,\mu$ on fibres of $20\,\mu$ external diameter to $1{\cdot}5\,\mu$ on the smallest medullated fibres. The axon of the large fibres becomes narrowed to about half its diameter as it passes through the node. The nodal membrane has an area of about $4\,\mu^2$ and varies little with fibre diameter.

The connective tissue sheath of a nerve as a barrier to the diffusion of phosphate ions. By G. CAUSEY and E. PALMER. *University College, London*

Feng & Gerard (1930) demonstrated a striking difference in the behaviour of frog nerve depending on whether its connective tissue sheath was intact or removed. Most subsequent workers have supported this finding, but it has been strongly criticized by Lorente de Nó (1950). These investigators assessed the permeability of sheathed and sheathless nerve by measuring the rate of onset of failure of conduction in soaked nerves. In the present work we have assessed the transfer of radioactive phosphorus in sheathed and sheathless nerves.

The epineurium (perifascicular connective tissue of Ranvier) can be removed easily as a continuous sheath. The perineurium (gaîne lamelleuse of Ranvier) remains on the nerve bundle.

The uptake of radioactive phosphorus was studied in the isolated epineural sheath and in the nerve without this sheath. If the nerve (either peroneal or tibial of the rabbit) was soaked in a radioactive solution before it was stripped the specific activity of the nervous tissue was low (7·5). If, however, the nerve was stripped before it was soaked the specific activity was increased more than tenfold (80). In either case the specific activity of the epineural sheath was very high (537).

It is concluded that *in vitro* the epineural sheath takes up the radioactive phosphorus and impedes the passage of phosphate ions.

Observations on the nature of peripheral influences producing maturation of regenerating muscle nerve fibres. By D. H. L. Evans. *University College, London*

It is well known that establishment of contact with the periphery exerts a marked influence on the pattern of nerve regeneration following cutting or crushing. Nerves not allowed to connect with a periphery and thus forming a neuroma regenerate a large number of fibres which remain small in diameter. Those allowed to reach their periphery develop a much smaller number of fibres which rapidly increase in diameter (maturation). The possible nature of this peripheral influence has been studied in rabbits by observing the effects of the following procedures on the regeneration of the nervus gastrocnemius medialis following crushing:

(1) The gastrocnemius muscle was tenotomized; maturation of regenerating nerves innervating these muscles occurred at the same rate as in intact muscles.

(2) Varying proportions of the muscle were removed; no effect was demonstrable on the rate of maturation as compared with intact muscles when as little as 8 % of the original muscle remained to be re-innervated. When the amount was less than this, however, marked retardation of rate of regeneration occurred.

(3) The fibres were allowed to regenerate along long and short pathways, composed of sural nerve. The distance travelled has no influence on regeneration, under the conditions of the experiment.

(4) Protein extracts of muscle were injected into the region of neuromas; these produced no demonstrable effect on regeneration as compared with injection of Ringer's fluid on the control side.

The effects of a foreign periphery on regeneration was investigated by comparison of the calibre spectra of intercostal nerves regenerating into their normal periphery; into a neuroma; and into the adrenal gland and anterior mesenteric ganglion. The latter periphery produces fibres which have a mean diameter intermediate between those innervating their own periphery and those forming a neuroma.

Early re-innervation of motor end-plates in partially denervated muscle. By D. B. Morris. *University College, London*

Since 1945, evidence has been forthcoming that surviving axons in partially denervated muscles may extend their field of innervation by the production of new fibres (Van Harreveld, 1945; Edds, 1950; Hoffman, 1951).

In the present study the partially denervated extensor digitorum longus of the rabbit has been examined histologically for evidence of new fibre production. This muscle is supplied by a large branch of the peroneal nerve, which divides into two distinct branches a short distance from its entry into the muscle. The upper division is usually the smaller, and this was divided between crushes and the stump implanted into surrounding muscle bellies. The muscles were examined from periods of 2 to 11 days post-operatively, staining was by a modified Bielschowsly technique using sections of 50μ thickness. Sections from 3- and 4-day muscles show: (1) intact sprays of terminal fibres and motor end-plates; (2) sprays with complete degeneration of all fibres; (3) sprays with one or few surviving fibres, the rest degenerated; (4) sprays with one or few degenerate fibres, the rest intact.

These findings can be explained on the basis that the motor unit is not confined to single groups of neighbouring muscle fibres, but innervates fibres scattered throughout the muscle.

New very fine fibres appear as early as 4 days and re-innervate denervated end-plates. The point of branching of the residual axons is very difficult to determine as they appear to come from far back in the nerve bundles. The process of collateral regeneration seems to be infrequent and not an extensive process as reported by some workers.

Regeneration in the vagus nerve. By J. G. MURRAY and D. H. L. EVANS.

University College, London

The operation of vagotomy, consisting of resection of 1–2 in. of the vagus nerve at the level of the diaphragm, has been widely used during the last 6 years in the treatment of duodenal ulcer. Recent reports on the results show that about 30 % of cases develop return of gastric hyperacidity within 6 months and a greater number within 18 months. There is little information available correlating these results with the histological findings in the peripheral vagal nerve trunks. Previous studies on vagotomy in experimental animals are few and present a conflicting picture.

In the present investigation the process of vagal regeneration is being investigated histologically, and an attempt made to correlate the findings with return of function. The abdominal vagi of rabbits have been subjected to crushing and to resection of varying lengths of nerve. Non-myelinated axons bridge the crush gap within 20 days and are also capable of regenerating across gaps of 15 mm. or more. In rabbits electrical stimulation of the cervical vagus produces a constant and well-marked increase in intra-gastric pressure which is always abolished by interrupting the vagi in the abdomen. After a simple crush there follows a period during which stimulation produces no effect or a *decrease* in intra-gastric pressure. This is gradually replaced by the normal rise in intragastric pressure which begins to appear about 130 days following the crush. The mechanism of the decrease in pressure associated with the early stages of regeneration is being investigated.

Following resection of a length of nerve, function returns within about 150 days but remains very incomplete, at least over a period of 300 days.

The organization of the visual cortex in the cat. By D. A. SHOLL.

University College, London

All methods for staining the cerebral cortex have disadvantages. Those of the Nissl type stain only the cell bodies of the neurones; the Golgi methods completely stain the processes of an unknown proportion of cells, while the silver methods cannot be used satisfactorily on sections thicker than 20μ since the preparations become opaque as a result of the large number of cell bodies, axons and dendrites that are stained.

A method of analysis has been devised that attempts to co-ordinate the quantitative observations obtainable from each of these three methods and form estimates of the nature of the organization of the nerve plexus at different depths of the cerebral cortex; the method has been applied to the visual cortex of the cat.

The total number of fibres, both axons and dendrites, in a unit volume is estimated by repeated samplings from sections stained by a silver method applicable to paraffin sections. Cell types at different depths are studied in Golgi-Cox preparations and the directions and diameters of all the dendritic branches of a number of cells measured. It was found that the distributions of the numbers of branches of the basal dendrites with respect to distance from the cell body are closely similar for each neurone. It is further shown that the number of these branches varies directly with the depth of the cell below the pial surface. Hence it is possible to estimate the numbers of branches at any distance from a cell if the depth of the cell below the pial surface is known. These results may then be transformed into the distribution of densities of basal dendrites per unit volume. Similar considerations apply to the apical dendrites and the contribution to the general dendritic density mady by any one cell may be found.

In order to assess the dendritic contributions to any particular element of volume of cortex, it is necessary to assess the contributions of all cells that may possibly have dendritic ramifications in that volume. To make this estimate it has been assumed that the cell bodies are arranged on a cubic lattice, the dimensions of this lattice being found from Nissl preparations. The difference between this total dendritic component and the total fibre estimates described earlier allow the axonal component to be estimated.

Finally, since the directions and diameters of the processes are known, not only the proportions of fibres proceeding in a given direction, but also the numbers of these fibres, axons and dentrites may be computed. This method has been carried out for different depths of the cat visual cortex, the axonal and dendritic densities per unit volume found and estimates made of the closeness of packing of the fibres. In this case it is found, for example, that in the lower part of the cortex a cortical volume of 10^4 μ^3 may contain dendrites arising from any of the cells from a total field of some 4000 cells, i.e. a cube of side about 20 μ.

The blood supply of the rat epididymis. By E. W. MACMILLAN.

University of Liverpool

The vascular pattern and its variations have been examined in twenty-one cleared specimens following injection with bismuth oxychloride.

The arteries primarily concerned are two in number, a superior and an inferior. They arise from the testicular artery about 1 cm. from the epididymis, either as two separate branches or by a common stem. The inferior vessel, on reaching the body of the epididymis, provides a branch which runs upwards towards the head (the ascending capital artery); the main trunk continues along the postero-medial aspect of the body, to reach the tail and form a well-defined anastomotic loop with the vasal artery. The terminal branches of the superior epididymal artery and the ascending capital branch of the inferior artery are constant sources of supply to the head and the adjoining portion of the body, but the inferior vessel may furnish one or two additional branches (the accessory capital arteries). Anastomoses between these various branches of the superior and inferior epididymal arteries occur in fifteen out of twenty-one cases.

Arterial ligations which occlude the superior epididymal artery and the accessory capital branches of the inferior artery, but spare the ascending capital branch, cause an enlargement of the testis detectable on the 3rd day and lasting until about the 7th day after operation when the testis begins to atrophy. Histologically, degenerative changes are found in the tubular system of the head and the vasa efferentia, and a disorganization of spermatogenetic function in the testis. Indian ink injection techniques have demonstrated that despite the dilation of intratesticular lymphatics the efferent lymphatic pathways remain open.

Further investigations are in progress to establish the long term effects of these experiments.

The vascularization of the rat pancreas. By D. J. ADAMS. *University of Liverpool*

The vascular pattern and its variations have been examined in twenty-one cleared specimens following injection with bismuth oxychloride suspension. There are four main arteries supplying the pancreas: (1) the superior pancreatico-duodenal; (2) the inferior pancreatico-duodenal; (3) the inferior pancreatic; (4) the splenic. Anastomoses are clearly present between the first three arteries in the head of the pancreas, but alternative channels of supply to the tail are not evident until after ligature and division of the splenic artery.

The superior pancreatico-duodenal, the inferior pancreatico-duodenal and the inferior pancreatic arteries unite with each other close to, and under cover of, the junctions of the 1st and 2nd, and 2nd and 3rd parts of the duodenum. Within the substance of the head of the pancreas filigree patterns and arcades are formed by slender branches of these arteries. Further anastomoses are thus provided which are presumably more effective after ligature and division of the main pathways.

The largest vessel to anastomose with the splenic is a tortuous artery running through the substance of the pancreas from the right gastro-epiploic at its origin and joining the middle or lower third of the artery. Additional subsidiary arterial anastomoses to the tail are provided from the left gastro-epiploic. Microscopical examination at intervals from 1 day to 16 weeks following interruption of the splenic artery at its origin suggests that these anastomoses are sufficient to maintain a normal histological picture. Experimental work on more profound degrees of ischaemia is being continued.

Pollex bifidus terminalis unilateralis *in vivo*. By M. A. MacConaill.

University College, Cork

The case showed a radial and an ulnar terminal element of the right thumb. The preliminary examination showed that there must be two proximal phalanges also, probably articulating with a single metacarpal. The radial element rotated during flexion, and could be adducted towards the ulnar element both in extension and in flexion. It was concluded that the radial metacarpo-phalangeal joint was saddle-shaped but that the corresponding ulnar joint was ellipsoidal ('normal'). These inferences were confirmed by radiography. The inferences were based on the author's thesis that sellar joints were more apt for conjunct rotation than ellipsoidal joints.

The insertions of the flexores digitorum profundus et pollicis longus, together with observations on the flexor pollicis sublimis. By J. L. Wilkinson. *University of Manchester*. (Introduced by G. A. G. Mitchell)

Investigation of fifty human thumbs and 100 fingers has revealed a striking difference between the disposition of tendon fibres of the flexor pollicis longus and the flexor digitorum profundus at and immediately prior to their distal insertions. This difference is also reflected in a comparison of their respective synovial sheaths. In view of the probable assimilation of a middle phalanx into the terminal phalanx of the thumb, it is interesting to note that, in a series of twenty-five dissections, the polliceal type of long flexor tendon insertion was noted in three little toes under the dual conditions of phalangeal fusion and an absence of the flexor perforatus tendon. The insertions of the long digital flexors were examined in a number of mammals, reptiles and amphibians. A *superficial* long flexor tendon, inserted into the terminal phalanx of the thumb, a condition common in some quadrupeds, was observed in a rudimentary state in certain primates. A connexion between the flexor digitorum sublimis and the terminal phalanx was noted by J. E. Frazer in the human foetus. There is a variable connexion between the flexors sublimis and pollicis longus in the adult forearm. The particular arrangement of tendon fibres in the human flexor pollicis longus insertion into the terminal phalanx, when considered alongside these observations, strongly suggests that an assimilated flexor pollicis sublimis tendon is associated with this insertion.

The development of the human mandibular joint. By N. B. B. Symons.

University of St Andrews, Dundee

This study is based on material from one embryo of 22 mm. c.r. length and from a series of foetuses (from 30 mm. c.r. length to full term). The condylar element of the joint is very early mapped out, and in relation to this the lateral pterygoid muscle seems to play some part. It is only after the appearance of the mass of secondary cartilage in the condyle that the previously widely separated mandibular and temporal elements become approximated, and it is through the rapid growth of the secondary cartilage that this occurs. Areas of secondary cartilage also appear in the temporal region of the joint, but these are smaller in size, appear later, and disappear before birth. The inter-articular disc appears to be derived, in part at least, from the tendon of the lateral pterygoid muscle; but is not produced by a compression of the tendon between the joint surfaces as they approximate, since a condensation representing the disc is present very early before the joint surfaces near each other and before any joint cavities have appeared. The connexion of the disc and lateral pterygoid muscle to the malleus has been confirmed and has been found to persist to at least the 180 mm. stage.

Two types of cardiovascular anomaly with comments on the so-called sinus venosus. By Franklin P. Reagan. *University of Birmingham*

Two types of congenital cardiovascular anomaly will be described as follows:

Case I. A condition associated with very low viability, and generally described as a 'common atrium'. The 'common atrium' receives right and left venae cavae superiores and also an inferior vena cava which is displaced to the left; there are extreme septal defects and a patent ductus arteriosus. In accounts of this condition the extreme suppression of the left atrium and the lack of communication between the pulmonary sinus and the left side of the 'common atrium' are usually overlooked.

It is suggested that this condition is due to a congenital failure of the normal torsion of the vestibulum atrii dextri (generally described as the sinus venosus in certain stages of development, though not in others), and to great proliferation of cardiac muscle in the hypertrophied left horn of this vestibulum. Evidence will be produced that the infravalvular and extravalvular portions of the vestibulum atrii dextri become absorbed into the caval rather than into the right atrium. The persistent foramen atriorum primum of the specimen is inferior to the lower sector of the limbus, a relationship which may suggest error in some of our developmental accounts.

Case II. A foetus showing slight terminal constriction of the coronary sinus with persistence of a small left anterior cardinal vein. There is also an anastomosis between the upper left pulmonary vein and the left innominate vein by which blood can be drained from the degenerating left anterior cardinal vein.

It is proposed to interpret this condition as being due to the development of precocious direct arteriovenous anastomoses between the hinder portion of the ventral aorta and one of the primordia of either anterior cardinal vein. From such anastomoses, capillaries are given off that communicate with capillaries around the developing respiratory tract. From such capillaries, the anomalous type of anastomosis described could conceivably develop. Injected specimens studied fail to show the early straight prominent intervenous anastomoses figured in wax reconstructions which form the basis for the customary interpretation of anomalous anastomoses between the end of the upper left pulmonary vein and the left innominate vein. Arteriovenous anastomoses as described are found in injections of early embryos of chick, rat, rabbit and bat.

Both anomalies indicate factors determining the fate of the left horn of the vestibulum atrii dextri.

Transuterine migration of the ovum in the albino rat. By A. Young. *The University, Glasgow*

The Fallopian tube on one side was ligatured in seventeen adult females who were thereafter mated. Seven of these females later showed evidence of implantation of ova in the uterine horn of the operated side. Histological examination showed that closure of the Fallopian tube had been achieved and that implantation had occurred. These findings will be discussed in relation to the anatomy of the uterus and ovary in the albino rat.

FEBRUARY 1952

An ordinary meeting of the Society, for the Session 1951–2, was held on Friday, 22 February 1952, in the Department of Anatomy, Middlesex Hospital Medical School, Mortimer Street, London, W. 1. The President (Prof. W. E. Le Gros Clark) was in the Chair.

The following are the authors' abstracts of the papers read.

Allanto-chorionic differentiations in the carnivora. By E. C. Amoroso. *Royal Veterinary College, London*

One of the characteristic features of the placenta of the Carnivora is the presence of a 'border' and 'central' haematoma. Where these occur, the vascularized allanto-chorionic membrane develops villi which come directly in contact with maternal blood. The maternal haemorrhages vary in size and position. In the dog, for example, they occur regularly along the margins of the placental zone, but in addition, smaller haemorrhages take place into the substance of the placenta and form the 'green pockets' or central haematoma. In the cat and coati, supernumerary 'pockets' are sometimes found in the form of 'chorionic vesicles' that are entirely lined by trophoblast, and which in their gross shape and finer morphology closely resemble the structures found in the chorion of the lemurs (Hill, 1932, *Philos. Trans.* B, **221**) and of *Scalopus* (Mossman, 1939, *Proc. Zool. Soc. B*, **109**). These vesicles are variable in size, are connected with the central haematoma and contain a blood-stained coagulum in which the extravasated erythrocytes become mingled with glandular secretions and disintegrating glandular epithelium. Our observations on the chorionic vesicles of the cat and the coati support reasons adduced by Mossman for supposing that they constitute an apparatus obviously similar to the marginal haematoma.

Besides the chorionic vesicles, the paraplacental chorion is sometimes provided with circumscribed, lightly staining, raised areas that are related to the mouths of uterine glands. These vary from single, enlarged trophoblast cells in the cat to large accumulations in the dog that are arranged in the form of typical 'rosettes' such as are found on the chorion of the pig or other ungulates. No mention has been found in the literature concerning these bodies. However, it is hardly likely that they have been completely overlooked, since the foetal membranes of the dog have been the subject of active investigations for more than 120 years.

Development and carbohydrate metabolism in the liver of the chicken embryo.

By R. J. O'Connor. *Westminster School of Medicine, London*

Carbohydrate catabolism has been measured in the liver of the chicken embryo by micromanometric methods. There is a decrease during the 7th day of incubation, and at the same time glycogen (measured by histochemical methods) appears in the liver cells and increases in amount. At this time there is no alteration in the rate of growth of the liver but other investigators have shown the beginning of adult function. The relationship of carbohydrate metabolism to growth and differentiation is discussed.

The rate of prenatal development of the goat *Capra hircus*.

By W. R. M. Morton. *Queen's University, Belfast*

The rate of development in a series of timed embryos and foetuses of the domestic goat from the late blastocyst stage up to the 90 mm. c.r. length stage has been estimated by using the greatest antero-posterior length in the early embryo, and the c.r. length later, as indices of development. The degree of organogenesis and these measurements have been used for comparison of this rate with that of the sheep as taken from the date of Green & Winters (1945, *Tech. Bull. Univ. Minn. Agric. Exp. Sta. no.* 169), and it has been found that the goat develops during this period at a slightly slower rate than does the sheep. This is in accordance with the findings of Amoroso, Griffiths & Hamilton (1942, *J. Anat. Lond.*, **76**) on the comparative rates of segmentation of the eggs and early blastocysts of these two forms.

Mesonephric derivatives in the human kidney.

By B. A. E. Johns and F. R. Johnson. *University of Sheffield*

Since Schiller, in 1939, described ovarian tumours histologically resembling mesonephric glomeruli and tubules, several other authors have observed similar new growths in the ovary, testis, broad ligaments and uterine tubes. All these tumours have been of a malignant type and their origin has been ascribed to mesonephric rests in the various tissues.

The present paper gives an account of structures, which the authors believe to be of mesonephric origin, in an adult human kidney removed at operation. These structures closely resemble the epididymis in having a lumen which is lined by tall pseudostratified columnar epithelium bearing stereocilia and having basal cells, containing lipoid droplets, on the inner surface of the basement membrane. The structures do not appear to have direct communication with any other normal constituent of the kidney and their epithelia are surrounded by a layer of poorly differentiated connective and muscular tissues.

The kidney shows coarse lobulation of its surface and the presence of areas of stratified columnar epithelium in the minor calyces.

The significance of these findings was discussed.

The diameter of muscle fibres in the m. gracilis of the rhesus monkey in the normal animals, after anterior ramisection and after anterior poliomyelitis. By R. E. M. Bowden. *Royal Free Hospital School of Medicine, London*

The diameter of 200 muscle fibres was measured in each of nine samples of m. gracilis taken from healthy adolescent rhesus monkeys and ten samples taken from the operated side and seven taken from the unoperated side after anterior ramisection. Similar measurements were made in twenty-six muscles taken from twenty-five animals at times ranging from 2 days to 6 months after the onset of acute anterior poliomyelitis. The rate of decrease in diameter of fibres was measured in muscles which had been completely paralysed by anterior ramisection or by anterior poliomyelitis. No significant difference was found in the rate of atrophy in the two conditions. There was no evidence that compensatory hypertrophy of muscle fibres played a significant part in the recovery of untreated skeletal muscle within the first six months after the onset of anterior poliomyelitis. In two animals with incomplete denervation after anterior ramisection there was also no evidence of hypertrophy. The interpretation of these observations raises the queston of the mechanism underlying recovery of function in partially denervated skeletal muscle.

Observations on microglia: the relation between nerve cell damage and microglial reaction. By E. J. Field. *University of Bristol*

The relation between morphological changes in nerve cells and microglial reaction has been studied in rabies, herpetic encephalitis and pseudorabies (Aujewsky's disease). Rabbits have been used, except for one series of experiments with mice. The rabbits were inoculated by the cornea, sciatic nerve, masseter muscle or adrenal gland, and the mice intracerebrally. They were sacrificed at the first elevation of temperature or onset of neurological disturbance. Nerve cell changes were studied in paraffin sections of Zenker fixed material stained with Giemsa or haematoxylin and eosin. Microglia was impregnated by Penfield's method.

In rabbit rabies it was found that:

(1) Microglia reacts precociously showing definite changes at a time when nerve cells show little or no change.

(2) Microglial changes are already well advanced before the onset of neurological signs.

(3) Microglial changes are widespread in the segmental region broadly corresponding to the peripheral part inoculated and are not more intense in the immediate neighbourhood of nerve cells or blood vessels.

(4) The microglia of the molecular layer of the cerebellum is especially reactive; disturbance of co-ordination and equilibrium are a prominent feature of the onset of rabies in the rabbit.

In the mouse (infected intracerebrally) there has been on the other hand no microglial reaction other than an intense one around the track of the infecting needle. No explanation of this difference can at present be offered.

In herpetic infection microglia behaves rather differently, for there is no early generalized reaction in the brain. After corneal inoculation when a severe lesion is produced in the ventro-lateral part of the medulla involving the spinocerebellar tract and the descending tract of the fifth nerve, microglia in the medulla reacts only moderately and less violently than would be expected in the vicinity of the destructive lesion which is, however, infiltrated with large numbers of mononuclear cells of unknown origin. Even in advanced herpetic infection (and in Aujewsky's disease) microglial response is much less than in early rabies.

The significance of these findings for the problem of microglial function was discussed.

Electromyography of muscles of posture. By J. Joseph and A. Nightingale. *Guy's Hospital Medical School, London*

Recently it has been suggested that the upright posture in man is maintained by the 'inherent elasticity' of the muscles concerned with this function. This appeared to be unlikely in the case of the calf muscles, in view of the relation of the line of weight to the ankle joint and of the force which the calf muscles would have to exert in order to maintain the upright position. Owing to the probability of there being differences between individuals, and age and sex differences, groups of subjects are being studied. This is a first report and deals with the leg muscles in males age 18 to 28 years. Standard postures and standard positions for the surface electrodes were used. It has been found that in all twelve subjects investigated, the soleus is constantly active in a weight bearing limb and in seven of the twelve, gastrocnemius is also active. The suggestion that 'inherent elasticity' of the calf muscles maintain the upright position should be abandoned. Why some subjects apparently use soleus alone and some use both soleus and gastrocnemius is discussed.

Electromyography of the erectores spinae muscles in flexion of the lumbar vertebrae. By W. F. Floyd and P. H. S. Silver. *Middlesex Hospital Medical School, London*

This movement will be demonstrated by a film which shows a subject standing upright, bending forwards, and in various sitting postures. The visual part of the film is accompanied by a simultaneous record on the sound track of the action potentials from the erectores spinae in the lumbar region.

It is suggested that the use of sound pictures in this way provides an excellent method of demonstrating and teaching the action of muscles.

Observations on the function of the lumbrical muscles of the hand. By K. M. Backhouse and W. T. Catton. *King's College, Newcastle-upon-Tyne*

An assessment of the different functions attributed to the lumbrical muscles has been made experimentally, their activity being studied by (1) electromyography, (2) electrical stimulation; using for all experiments normal subjects under no form of anaesthesia.

It has been shown by electromyography that the lumbrical muscle is contracting powerfully only in movements involving extension at the interphalangeal joints, the position of the metacarpophalangeal joint being relatively unimportant. Action potentials could not be recorded from the muscle in metacarpo-phalangeal flexion without interphalangeal extension, nor in radial deviation or rotation.

Direct stimulation always led to extension of the interphalangeal joints, with secondary flexion of the metacarpo-phalangeal joints only when the stimulating current was high enough. There was no evidence of radial deviation or rotation.

It is thought, therefore, that the principal action of the muscle is that of an extensor of the interphalangeal joints, assuming that both the long muscles upon which it operates are in a normal functional state. It is a weak flexor of the metacarpo-phalangeal joint, acting if the interphalangeal joints are extended. Its value as a radial deviator or rotator is extremely doubtful.

Locking at the knee-joint. By C. H. Barnett. *St Thomas's Hospital Medical School, London*

There are at least three aspects of the terminal locking mechanism at the knee-joint which require consideration: first, the phase of extension at which medial rotation of the femur commences; secondly, its functional significance; and, thirdly, the factors responsible for the movement.

Cine-radiographic studies indicate that although rotation of the femur is initiated while the knee is at least 30 degrees short of full extension, the greatest movement occurs during the last 10 degrees, the phase in which, during walking, there is maximal pressure between the articular surfaces.

MacConaill (1946) has shown that at any hinge-joint there is an element of axial rotation which reduces the frictional wear of the articular cartilage, and it is therefore probable that the locking movement not only serves to 'screw home' the knee in full extension, thereby increasing the stability of the joint, but also has an important function in reducing the friction at the joint surfaces throughout the weight-bearing phase of walking.

In the normal knee-joint, the contours of the articular surfaces are of considerable importance in producing rotation of the femur during extension, although several other factors undoubtedly contribute. In a series of twenty normal knees examined in fresh amputation specimens and preserved cadavers, it has been found that the articular surfaces of the *lateral* femoral and tibial condyles become congruous well short of the fully extended position; at this stage the *medial* condyles are still incongruous and thus in the phase of mobility (Walmsley, 1928). Further extension of the lateral condyle is now impossible, and in these circumstances contraction of the quadriceps muscle brings about medial rotation, turning the femur about an axis that passes through the head of the femur and the lateral femoral condyle. This rotation has the effect of freeing the lateral articular surfaces from their 'close-packed' state, permitting the femur to undergo further extension, which will continue until these surfaces again become congruous. The process of alternating extension and medial rotation is repeated until extension is complete. At this stage both the medial and the lateral femoral condyles are fully congruous with the corresponding tibial surfaces, and the joint is locked.

The shoulder mechanism of reptiles. By R. Wheeler Haines. *University of Sheffield*

In modern lizards the shoulder joint is provided with four strong ligaments arranged symmetrically in two cruciate pairs, allowing the humerus some freedom of movement in all directions. In early tetropods the joint surfaces were elongated and twisted in a way no longer found in any living form, but the experimental attachment of lizard-like ligaments to a model of the extinct reptile *Dimetrodon* gives a workable joint and explains some of the peculiarities of the bones, and is compatible with what is known of the gait of early tetrapods from preserved tracks. It is suggested then that the modern lizards have preserved the primitive set of ligaments in spite of great modifications of the joint surfaces, but that the ligaments have been lost in the line leading to the mammals, where the mechanism of the gait has changed.

Abnormal disposition of the pulmonary veins. By H. Butler. *St Bartholomew's Hospital Medical College, London*

Complete or incomplete drainage of the pulmonary veins into the right atrium or into the systemic veins is sufficiently uncommon, but even rarer is their entrance into the portal venous system. A survey of the literature reveals reports of 133 confirmed cases since the first was described by Winslow in 1793 (Brody, 1942; Young, 1947; Edwards & DuShane, 1950). Twelve of these showed abnormal pulmonary veins draining, totally or partially, into the portal venous system; in three instances only did the abnormal pulmonary vein do so via a still patent ductus venosus (Ghon, 1916; Mehn & Hirsch, 1947; Edwards & DuShane, 1950). The specimen here described constitutes a fourth example of this latter type of anomaly.

The pulmonary veins join to form a common pulmonary trunk, 2·5 cm. long and 4·0 mm. wide, which runs caudally along the posterior surface of the oesophagus to perforate the diaphragm through a discrete foramen situate between the oesophageal and the caval foramina. It then runs in the groove between the caudate and left lobes of the liver to terminate in the left branch of the portal vein immediately to the left of the still patent ductus venosus. Its terminal orifice is considerably narrowed by proliferating, vascular connective tissue such as is responsible for the normal obliteration of the ductus venosus. As a result of diminished entry of blood the left atrium is reduced in capacity to about half that of the right atrium, and there is a corresponding reduction in left ventricular capacity.

It is believed that the pulmonary vein is of dual developmental origin (Streeter, 1945; Auer, 1948; and others), and on the basis of such an hypothesis a possible explanation of this anomaly is offered.

The flexor digitorum brevis muscle in the foot of the South African negro.

By L. H. Wells. *University of Edinburgh*

This muscle has been examined in about four hundred specimens and found to be remarkably variable. A deep lamina arising from the long flexor tendons and distributed to one or more toes was present in 52 %. The 'normal' or superficial lamina supplied tendons to all four lateral toes in less than 40 %. There is great variation in the pattern of formation of the digital tendons. The predominant trend is towards replacement of superficially arising by deeply arising tendons to the more lateral digits, paralleling the condition in the monkeys and apes, with a subsidiary trend towards a completely bilaminar arrangement which appears to be the primitive mammalian condition. There is also a tendency for the tendon to the little toe to be completely suppressed. Such data as are available indicate that this muscle is more variable in the South African negro than in other types of man. This may be an expression of a genetic tendency to muscular variability in the South African negro, but it is also possible that it may be related to special features in the foot mechanism.

Variations in origin of the parietal branches of the internal iliac artery.

By J. L. Braithwaite. *University of Liverpool*

The sites of origin of the parietal branches of the internal iliac artery have been investigated in British subjects, 169 pelvic halves (108 male and 61 female) having been studied. The variations in origin of the superior gluteal, inferior gluteal, and internal pudendal arteries have been grouped into four 'types', according to Adachi's (1928) modification of Jastschinski's (1891) method.

Type I, in which the superior gluteal arises from the internal iliac artery proximal to a common trunk for the inferior gluteal and internal pudendal vessels, is found in 58·5 % of specimens.

Type II, where a common stem gives origin to the gluteal arteries proximal to the origin of the internal pudendal artery, in 15·3 %.

Type III, in which the three vessels arise separately, in 22·5 %.

Type IV, in which all three vessels arise by a common trunk, in 3·6 %.

In 55 % of instances these arteries exhibit similar origins on both sides of the pelvis.

The obturator artery is too variable to be used as a factor in 'typing'. It arises as a direct branch from the anterior division of the internal iliac artery in 41·4 % of instances, from the inferior epigastric in 19·5 %, from the superior gluteal in 10 %, from the inferior gluteal-internal pudendal trunk in 10 %, and by a double origin in 6·5 %. Previous findings give a higher incidence for an inferior epigastric origin, and a lower for a double origin. In only 23 % does the obturator artery arise in similar fashion on both sides.

A comparative study of neuron types in the mammalian neo-cortex.

By N. L. Mitra. *University College, London*

The present study is a report of twenty-six sets of observations made on different functional areas of the cortex of rabbits, cats and monkeys. Frequency distributions of the various types of neurons in relation to depth, measured from the pial surface, have been made from sections $160\,\mu$ thick stained by the Golgi-Cox method. A limited investigation has also been made of the frequency distribution in the primary visual area of animals at various ages.

The results indicate that in spite of the selectivity of the staining method, samples from the same animal lead to statistically consistent results. The neurons can be classified into pyramidal, stellate and spindle-shaped forms, but the latter are only found in considerable numbers in those regions of the cortex with a high curvature. A concentration of stellate cells is found in the primary receptive areas of the cortex, being closely related to the termination of specific afferent fibres. Elsewhere they are distributed throughout the whole depth of the cortex. There also appears to be an increase in their number as an animal approaches maturity. The progressive increase in the proportion of stellate cells and their more restricted distribution in the series of animals, rabbit, cat and monkey, suggest a connexion with the development of visual function.

Residual cells in the hemidecorticate human thalamus.

By T. P. S. Powell. *University of Oxford*

A study of the retrograde cell degeneration in the human thalamus after hemidecortication shows that cells with only slight changes in staining properties and in the appearance of the Nissl substance persist in the ventral nuclei. These cells constitute 40–50 % of the normal cell population, and the majority are large cells and uniformly distributed throughout the ventral nuclei. The dorsomedial nucleus is completely degenerated except for the magnocellular element and small groups of cells related to a limited area of orbital cortex left intact. There is no evidence of any change in the midline and intralaminar nuclei. The centre median and reticular nuclei show partial cell loss. It is suggested that the residual neurons in the ventral nuclei either do not project on to the cerebral cortex or they send strong collaterals elsewhere.

APRIL 1952

An ordinary meeting of the Society, for the Session 1951–2, was held on Friday, 25 April 1952, at the London Hospital Medical College, Turner Street, London, E. 1. The Vice-President (Prof. D. V. Davies) was in the Chair.

The following are the authors' abstracts of the papers read.

The foetal placenta and membranes of *Halichoerus grypus*.

By R. J. Harrison, E. C. Amoroso and L. Harrison Matthews. *London Hospital Medical College, Royal Veterinary College and Zoological Society, London*

A number of specimens of the shed placenta and membranes of the Grey Seal (*Halichoerus grypus*), and several near-term pregnant uteri of the Common Seal (*Phoca vitulina*) have been examined. A description was given of the arrangement of the membranes with particular reference to the large tent-like yolk sac. The latter is almost pyramidal in form with its four main limbs spreading out over the placental band and extending for a short distance on to the paraplacental region. The chorio-allantoic vessels are invaginated into the yolk sac and injection of the vessels has shown that they provide an extensive vascular network of fine vessels to the yolk sac wall. In the specimens from *Halichoerus grypus* numerous cord-like strands of firm material were found within the yolk sac, and were connected to the yolk sac wall by slender tendon-like processes. Evidence is presented which suggests that these strands are remnants of the chorio-vitelline vessels which have become included within the yolk sac.

The placental labyrinth consists of numerous convoluted lamellae, enclosing the thin-walled maternal capillaries; the lamellae are arranged more or less in lobules. The junctional zone is characterized by a proliferation of the syncytium and by greatly dilated maternal glands. Marginal and central haematomata, brick-red or brown in colour are constantly present, the central haematomata being usually in relation to the edge of the yolk sac, but occasionally at the opposite pole of the placental girdle. Vascularized and convoluted chorio-allantoic villi are found in the haematomata. Well vascularized 'chorionic vesicles' of varying size have been found in all specimens of *H. grypus*, usually at the base of the yolk sac and lying in relation to central haematomata. Small, discrete, yellow plaques 2–5 mm. in diameter, lying beneath the allantois, are to be seen distributed over the foetal aspect of the placental band; their nature and significance was discussed. The structure of the paraplacental chorion and its blood supply was described.

Development and growth of the placentomes in the Cervidae. By W. J. Hamilton and R. J. Harrison. *Charing Cross Hospital Medical School and London Hospital Medical College*

A collection of a large number of pregnant uteri of several species of Cervidae has enabled a description to be made of the method of formation and growth of the placentome (maternal caruncle and foetal cotyledon). Specimens of *Cervus elephas*, *C. nippon* and *Dama dama*, pregnant with embryos varying in length from 10 to 300 mm., were compared as regards the arrangement of the foetal membranes, the number and shape of the placentomes and the manner of their increase in size. Comparison was also made of the types of villi found in the different species, and the manner of their invasion of the maternal caruncle. Some of the specimens have been injected with coloured substances and descriptions were given of the development of a network of maternal capillaries surrounding the crypts containing the villi. The development of the intra-epithelial capillaries of the foetal villi does not occur until the placentome is well established and has a profuse maternal blood supply. The inter-cotyledonary part of the chorion with its areolae was described in the various species.

Distribution of radio-potassium in the uterus of the pregnant rat. By J. L. D'Silva and R. J. Harrison. *London Hospital Medical College*

The distribution of radio-potassium in the uterus, in the placentae and membranes, and in the foetuses has been studied after intravenous or intraperitoneal injection of 1–16 mg. of ^{42}KCl into albino rats pregnant for 19–20 days. The rats were bled from the carotid artery at times varying from 30 sec. to 24 hr. after injection. The uterus, different parts of the placentae, and foetuses and a maternal kidney were quickly removed, weighed and dissolved in nitric acid. The radio-activity of the digests was determined and the results expressed, after correcting for decay, as counts per gram of tissue per minute calculated as a percentage of the total counts injected.

The results show that the labyrinthine placenta of the rat is highly radioactive 30 sec. after injection of ^{42}KCl and that 5 min. after injection its radio-activity per gram of tissue is one-eighth of that of the maternal kidney. After 24 hr., equal weights of the maternal kidney, the labyrinthine placentae and the foetuses are nearly equally radio-active. The maternal kidney is about one-thirteenth as active compared with the distribution at 5 min. after injection, but the radio-activity of the labyrinthine placenta has only fallen by about a half. The yolk-sac placenta (vascular splanchnopleur) is only one-tenth as active per gram of tissue as the labyrinthine placenta 15 min. after injection. The uterus, after removal of the foetuses, foetal placentae and membranes, has an activity per gram of tissue of an order comparable with that found in the uterus of non-pregnant controls. After 5 min., 0·032 % of the radio-active material injected was present in each gram of foetus, and

0·326 % was present after 24 hr. This implies a net accumulation of radio-potassium per gram of foetus in 24 hr., and suggests that some of the foetal tissues exchange potassium relatively slowly. On the other hand, the findings indicate that radio-potassium exchanges very rapidly with some cellular constituent of the labyrinthine placenta of the rat. Evidence from histochemical investigations and autoradiographs on the distribution of the potassium in the labyrinthine placenta was discussed.

The mammalian mandible. By F. Wood Jones. *Royal College of Surgeons, England*

From a re-examination of the conditions prevailing in submammalian vertebrates, it is suggested that the dorsal extremities of the two most cephalic pharyngeal bars, which are in proximity to the otocyst, are, from their first inception, associated with otocyst function. This function is retained, with various modifications, throughout the whole of the vertebrate series. The mammals differ from all the submammalian vertebrates in that many of them prey on creatures larger than themselves and all of them bite up and chew their food before swallowing it. This change of function demanded a change of structure in the form of a differentiated dental series and an altered type of mandible and temporo-mandibular articulation. But the old structural and functional association of the dorsal elements of the mandibulare and hyale with the otocyst—and later with the tympanic cavity—remained undisturbed.

The mobility of the fibula in eutherian mammals. By C. H. Barnett and J. R. Napier. *St Thomas's Hospital Medical School, London*

The mobility of the fibula has been studied in the hind-limbs of ninety-two eutherian mammals, including twenty-two wet specimens. On the basis of the anatomy of the tibiofibular articulations it is possible to classify the majority of fibulae into three main types:

(1) The *immobile* fibula, partially fused to the tibia.

There are two main varieties: (*a*) a thin, flexible fibula, with fused inferior and synovial superior tibiofibular joints—typically found in limbs capable of sudden movements, as in saltatorial mammals; and (*b*) a stout, inflexible fibula usually fused with the tibia at both ends—typically found in fossorial and aquatic mammals.

(2) The *mobile* fibula, with synovial tibiofibular joints. This type is found in certain carnivores and the majority of primates and appears to be associated with the ability to adapt the foot to uneven surfaces. The principal movement of the fibula is a lateral rotation as the foot is dorsiflexed.

(3) The *intermediate* type of fibula, articulating with the tibia by a synovial joint superiorly and a syndesmosis inferiorly. This type is found in mammalian limbs that approximate most closely to the primitive pattern, as well as in those species showing some modification towards a specialized mode of progression.

A tendency towards disappearance of the fibula is a cursorial specialization.

The occurrence of identical tibiofibular articulations in diverse species does not necessarily indicate a direct ancestral relationship, but rather a specialization towards a common mode of locomotion.

Movements of the sacro-iliac joints. By H. Weisl. *University of Manchester*

Movements of the sacro-iliac joints were investigated, using lateral radiographs of pelves of living subjects.

Fixed points on the sacrum and the innominate bone allowed comparison of the radiographs. The accuracy of the method was checked by a series of twenty-seven control experiments; in over half of these the results were accurate to 1 mm.; in none did the variation exceed 4 mm. This agreed with the expected variation determined mathematically.

A hundred and eleven young adults of both sexes were then examined. In 63 % the true conjugate diameter of the pelvis remained unchanged during full trunk extension; in the remainder the numbers showing increase or decrease in the diameter were approximately equal. In trunk flexion 54 % showed no change and 37 % had a significant reduction in the conjugate diameter. Following trunk and hip flexion combined, the diameter was reduced in 50 % of the subjects.

During extension the sacral promontory remained stationary or moved slightly dorsally or ventrally; this accounted for the variations in the true conjugate diameter. During flexion the promontory moved ventrally in 58 % and in the others it remained stationary or moved dorsally.

No movement was detected in less than 10 % of the subjects.

In 60 % of the experiments a position for a putative, geometrical centre of rotation could be determined; it was approximately 10 cm. below the sacral promontory.

Regulation of the choroidal circulation in the dog and cat.

By D. B. MOFFAT. *University College, Cardiff*

The blood supply of the retina in the dog and cat is by means of branches of the posterior ciliary arteries. These arteries in the dog are characterized by the presence of subendothelial 'cushions' consisting of bundles of longitudinally running smooth muscle fibres. The cushions first appear in the artery as it begins to run forward through the sclera alongside the optic nerve, and they cease to be present shortly after the artery has turned into the choroid. They are absent from the smaller branches which run more obliquely through the sclera without giving a retinal branch, although the points of origin of these vessels from the main artery are often guarded by cushions. In many instances, a cushion splits to enclose the mouth of a chorodial branch and then reunites, thus giving a valve-like appearance in cross-section.

In the cat, the cushions are fewer and smaller in the main vessels, but are almost invariably present at the point where the retinal vessels are given off, where they resemble the 'bourrelets valvulaires' of Legait. The muscle cells in these cushions are often rounded and epitheloid in appearance.

The possible functions of these structures were discussed.

The effect of cortisone and vitamin C upon the tensile strength of wounds in rabbits.

By G. H. BOURNE. *London Hospital Medical College*

Various authors have shown that cortisone has an effect on the connective tissue of animals similar to that brought about by vitamin C deficiency. Since lowered tensile strength has been found in wounds in the latter condition, wound strength has been tested in cortisone injected rabbits and in rabbits injected both with cortisone and with vitamin C.

Cortisone caused a reduction of tensile strength in an experimental wound of between 30 and 50 %. Daily doses of 1·0 g. of vitamin C per rabbit failed to prevent this fall significantly.

The effect of quinine on the ribonuclease reaction of tissues.

By A. F. BARADI and G. H. BOURNE. *London Hospital Medical College*

It has been shown histochemically that quinine increases the ribonuclease reaction in the gustatory epithelium of the rabbit. Since this enzyme is present in many other tissues, the effect of adding 0·5 % quinine to the substrate used for demonstrating ribonuclease elsewhere was investigated.

In rat tissues quinine was found to cause a varying degree of intensification of the ribonuclease reaction in the heart, liver, adrenal, small intestine, lung, salivary glands, lymph nodes, pyloric stomach, trachea, pancreas, parotid gland, ovary and skin. Quinine was found to have no effect on the ribonuclease reaction in the kidney, thymus and large intestine.

In rabbit tissue addition of quinine to the substrate caused intensification of the reaction in the testis, epididymis, adrenal, oesophagus, trachea, thyroid, spleen, duodenum, small intestine, kidney, heart and lung. There was no effect on the reaction in the pancreas and thymus.

Quinine appeared to accentuate the reaction in the nuclei more than in other parts of the cell.

The distribution of simple esterases in tissues. By H. A. Malaty and G. H. Bourne. *London Hospital Medical College*

Simple esterase is present in most of the tissues of the body. It is an enzyme which splits simple esters such as naphthyl acetate and differs from lipase which splits long chain fatty acids and from choline esterase. Nevertheless, there is evidence that this esterase may have some ability to split these two latter enzymes and that it may also split a number of related simple esters. Another view is that there is a series of these simple esterases each with a varying amount of substrate specificity. The present work has been carried out with the object of shedding light on this problem. At the same time opportunity has been taken to study the diazo-coupling reaction which is the basis of the method for demonstrating esterases; for this purpose six azo dyes of the Brentamine series were used, and it was found that some of these are more active couplers with some substrates than with others, and it was obvious that in an investigation of the reaction in a tissue with a particular substrate it was necessary to have the right azo dye to go with it.

Using three different substrates (α-naphthyl acetate, β-naphthyl acetate and naphthyl A.S. acetate) with appropriate azo dyes, differences of distribution were found in various organs which suggested that more than one esterase was present. The organs used were kidney, liver, lung, duodenum, cerebrum, cerebellum.

The origin of islet cells in the pancreas. By H. Hughes. *University College, Cardiff*

A series of fourteen hooded rats received each a single dose of alloxan and were killed at intervals thereafter ranging from 15 min. to 10 days. The pancreas was removed and the Golgi net of its cells impregnated by Aoyama's method.

The characteristic net of the α-cells remained unchanged in all the animals. The net in the β-cells of those animals killed shortly after the administration of alloxan failed to impregnate. In animals killed at longer intervals after the administration of the drug the Golgi material reappeared in the form of globules scattered irregularly throughout the cells. In animals allowed to survive for some days a greatly hypertrophied net could be observed.

The net in acinar cells bordering the islets exhibited similar but less intense changes, while in acinar cells at some distance from the periphery of the islet the net was unaffected by the drug.

These findings appear to be compatible with the hypothesis that acinar cells are constantly being transformed into islet cells.

Histochemical studies of autografts and homografts of human skin. By R. J. Scothorne. *University of Glasgow*

In a previous communication to the Society (1951), the changes in glycogen and ribonucleic acid content of human skin autografts were reported. The study has since been extended to two further cases, in which split skin autografts and homografts were used. Up to 10 days after grafting, autografted and homografted epithelia behave similarly in respect of glycogen and ribonucleic acid. At 15 days, however, the homografted epithelium is breaking down. Its cells are swollen and contain little or no ribonucleic acid, and the nuclei are pyknotic. Glycogen is still present in some of the cells. At 20 days the autografted epithelium remains healthy, and still contains glycogen. The homografted epithelium has disappeared. The significance of these findings was discussed.

JUNE 1952

The Summer Meeting of the Society, for the Session 1951–2, was held on Friday and Saturday, 27 and 28 June 1952, in the Department of Anatomy, University of Manchester. Profs. J. C. Brash, D. V. Davies and G. A. G. Mitchell were in the Chair at the various sessions.

The following are the authors' abstracts of the papers read.

The rostral extremities of the sympathetic trunks. By G. A. G. Mitchell. *University of Manchester*

The superior cervical ganglia are uniformly described as the rostral extremities of the sympathetic trunks and the internal carotid nerves as branches of distribution. All the ancient anatomists believed the trunks entered the skull and were branches of the vagus or abducent nerves. They were mistaken in the latter belief, but were they wrong in the other? Perhaps not. The internal carotid nerves are normally as thick as the trunks between the superior and middle cervical ganglia and, much more significant, they always contain varying-sized collections of ganglion cells; these have been found at all levels up to, and including, the plexuses around the anterior cerebral arteries.

It is suggested the internal carotid nerves are cephalic portions of the sympathetic trunks, and that the contained ganglia belong to the primitive head segments which have become telescoped and fused in the chordal part of the skull base; evidence of segmentation persists in the myotomes, branchial arches and mode of innervation.

If this view is correct, then the rostral extremities of the sympathetic trunks are interconnected by filaments alongside the anterior communicating artery, and a macroscopic ganglion has been described in this situation, although one has seen only microscopic collections of cells: the analogy with the caudal arrangement of the trunks is obvious. A proportion of the fibres in the internal carotid nerves are preganglionic to the ganglia on their course, and not all postganglionic as usually stated, and some of their branches are rami communicantes passing to various cranial nerves.

An experimental study of the origin of parasympathetic fibres to the ciliaris oculi and sphincter pupillae. By R. Warwick. *University of Manchester*

Considerable evidence exists to show that the innervation of the ciliaris oculi and sphincter pupillae is by means of a pathway in the oculomotor nerve, with a relay in the ciliary ganglion. Little of this evidence concerns primate animals, however, and the midbrain source of these autonomic fibres remains uncertain. Most texts and monographs accept the Edinger-Westphal nucleus as the autonomic component of the oculomotor complex; but some authorities have denied this.

Using a retrograde degeneration technique the pathway of these fibres was studied in monkeys (*Macaca mulatta*). Divisions of the whole nerve, resection of the ciliary ganglion, division of the short ciliary nerves, enucleations, iridectomies, and evacuation of the eyeball were carried out. The results confirmed that the nerve supply of the ciliary muscle and sphincter of the pupil are supplied in monkeys by fibres originating in the Edinger-Westphal and antero-median nuclei and relaying in the ciliary ganglion. The great majority of the post-ganglionic fibres reach the ciliary muscle.

The arterial supply to the stellate ganglion. By E. L. Patterson. *University of Manchester*

Various branches of the subclavian artery have been mentioned as contributing, constantly or variably, to the blood supply of the inferior cervical and first thoracic sympathetic ganglia, either as separate ganglia or united as the stellate ganglion. There is, however, an absence of detailed information about the ganglionic arteries. The sources and arrangement of these vessels have been examined in infants after arterial injection.

The principal supply is constantly derived from the branches of the costocervical trunk, especially the superior intercostal artery, but there is usually a supplementary supply from other nearby arteries, such as the inferior thyroid, thyro-cervical trunk, subclavian (directly), first aortic intercostal and internal mammary, one or more of which commonly contribute. The ganglionic arteries may, in general, be classified into lateral, posterior and medial groups.

An investigation of the factors controlling silver staining.

By E. P. SAMUEL. *University of Manchester*

The investigation showed that the staining of nervous tissue is partially controlled by the pH and silver concentration of the impregnating solution and the time and temperature of the reaction and that all these factors act in an interdependent manner. Silver concentration and pH alone do not determine 'specific' staining as has been previously believed. When immersed in a buffered silver solution two types of silver are deposited in the section: (*a*) a fraction of silver reduced by the section as minute silver particles, i.e. silver 'nuclei', which in a given time decrease in number as the pH rises; and (*b*) a fraction of silver which is reduced by the developer, i.e. 'reducible silver', which in a given time increases with increasing pH of the buffer. The silver 'nuclei' do not appear to be simply centres for the deposition of the reducible silver when the section is placed in the developer as Liesegang (1911, *Kolloidchem. Beih.* 3) believed, for under certain conditions there may be no correlation between the staining on development and the deposition and amount of the 'nuclei'. It is suggested that they act as centres for the catalysis of silver reduction. In every investigation a transient equilibrium occurs between the silver nuclei and the reducible silver and the impregnating solution. As the impregnation is prolonged there is a tendency for the larger 'silver nuclei' to increase at the expense of the smaller ones and a consequent variation in staining ensues. This occurs more rapidly at high pH levels. The investigation revealed that the properties of the developer play a fundamental role in the final distribution of the silver and cause a profound alteration in the staining effects.

Some observations on the innervation of the cat's hind-limb. By A. JEFFERSON.

University of Manchester. (Introduced by G. A. G. Mitchell)

In the course of studying the reflex activity of the cat's spinal cord, in terms of the electrical discharges present in the ventral root, we have found it necessary to examine the exact distribution of nerve fibres to and from the individual muscles of the hind-limb. This is necessary for the correct interpretation of reflex discharges present in individual ventral roots.

In attempting to plot the distribution in muscle nerves we have used some of the conventional methods of anatomy and of physiology—that is to say histological examinations and the recording of action potentials in nerve fibres.

The innervation of the hamstring muscles and of the musculature of the lower leg has been mapped and the methods and results are described. It would appear that the physiological method of identifying the distribution of nerve fibres has considerable possibilities at least as a preliminary to histological studies. There are limitations in the usefulness of studying surviving fibres arising from individual ventral roots because of the fact that the plexus may not be normally 'fixed'. However, some information concerning the intraneural distribution of the motor fibres of individual segments has been obtained and is described.

The 'lobule désert' and anterior pyriform area of the porpoise (*Phocaena phocaena*). By A. S. Breathnach. *St Mary's Hospital Medical School, London*

Through the co-operation of Dr E. Ford of the Marine Station, Millport, Scotland, two well-fixed specimens of the brain of the porpoise, *Phocaena phocaena*, were obtained. Since most previous work on the 'olfactory centres' of the anosmatic and microsmatic Cetacea (Addison, Jelgersma, etc.) is confined to the study of Weigert preparations these specimens have been used to study the cellular pattern of these regions.

On the ventral aspect of the brain of *Phocaena* is a prominent region—the 'lobule désert' of Broca—which bears a superficial resemblance to the olfactory tubercle of other mammals; it is bounded anteriorly and laterally by a gyrus, limited by well-defined medial and lateral sulci, neither of which, it is suggested, corresponds to the rhinal fissure. In Nissl preparations, three major fields can be outlined on the surface of the 'lobule désert'—(1) the head of the caudate, (2) a formation closely resembling in structure the olfactory tubercle of macrosmatic mammals, and (3) the nucleus of the diagonal band. The characteristics, extent, and relations of these areas are discussed and illustrated. Along the medial edge of the gyrus anterior and lateral to the 'lobule désert', a narrow strip of cortex which shows the characters of 'allocortex' can be distinguished. It is suggested that this represents the anterior pyriform area of *Phocaena* and that the morphological position of the rhinal fissure lies along its lateral limit. The bearing of these findings on previous interpretations of the position of the rhinal fissure in the Cetacea is discussed.

Effects of cortisone compound E and compound A on the bone marrow of young male guinea-pigs. By J. M. Yoffey, R. J. Ancill, J. A. G. Holt and B. Owen-Smith. *University of Bristol*

The work has been performed on seventy-seven young male guinea-pigs, about 3 months old. Thirty animals were used as controls, thirty were treated with compound E, seven with compound F, and ten with compound A. Experimental animals were given daily injections of 5 mg. of the steriod compound, suspended in a special medium, for 7 days; the controls were given injections of the suspending medium only. The marrow was examined by the quantitative technique previously described.

There seemed to be no difference in response to compound E (cortisone) and compound F (hydrocortisone). After both these substances the marrow was more cellular than normal, as indicated by an increased absolute count; the increase was especially marked in the erythroid series. There was no significant change in the marrow eosinophils or lymphocytes. Compound A, on the other hand, seemed to act as a more direct stimulus to granulocyte formation, and granulocytes appeared to be forming directly from reticulum cells, with what appeared to be a short-circuiting of the myeloblast stage.

The skull and reputed busts of William Burke the notorious murderer. By J. C. Brash. *University of Edinburgh*

The authentic skull of William Burke has been compared by the photographic superimposition technique with the cast of the head taken by Mr Samuel Joseph, the Sculptor, before the public dissection on the day after the execution (Thursday, 29 January 1829). It has been compared also with another cast (of which there are several copies) which differ in some details. The proportions of the former correspond to those of the skull but its dimensions are in excess of expectation, probably owing to the method of hanging. The latter cast appears to be a copy taken indirectly from the former by a process of free moulding which accounts for the variations in detail.

The morphology of the costo-clavicular ligament. By A. J. E. Cave and R. Warwick Brown. *St Bartholomew's Hospital Medical College, London*

The constitution of the human costo-clavicular (rhomboid) ligament has been reviewed in amplification or emendation of the generality of current descriptive accounts: its anatomy has also been studied in a series of non-primate and primate mammals, with a view to the determination of its morphology.

The human structure has the form of a short, inverted, truncated cone, with continuous anterior and posterior laminae which enclose a constant and discrete cavity. This cavity may be smooth-walled, obvious and fluid-filled, or, more commonly, much obscured by multiple fibrous strands and minute fatty lobules. Medially the ligament abuts against the sterno-clavicular capsule; laterally (rarely anteriorly) lies the subclavius tendon, frequently grooving the ligament but never continuous with it.

Topographical and comparative considerations demonstrate clearly the nature of the rhomboid ligament as a portion of the sterno-clavicular joint capsule specialized to form a functional inferior ligament of that articulation and guaranteeing its integrity: they refute the notion (Bland Sutton, 1897) that the rhomboid ligament is morphologically a metamorphosed portion of the subclavius tendon.

The development of the femoro-tibial joints with a discussion of the alleged femoro-fibular articulation. By R. Wheeler Haines. *University of Sheffield*

Cardboard reconstruction models of the knee at the period of interzone formation allow a detailed description of the articulations. In a 13 mm. embryo though interzones are found in the arm and at the hip between the ilium, ischium and femur, the cartilage of the tibia has not yet approached sufficiently closely to the femur for an interzone to be formed. By 16 mm. dense interzones have been formed between each condyle of the tibia and the corresponding condyle of the femur, and later these become three-layered and finally dehisce. But contrary to certain reports in the literature, neither at this stage nor at any other is an interzone formed between the fibula and the femur. The early development of a joint capsule cutting off a mass of synovial mesenchyme in which the cruciate ligaments develop later is confirmed in the knee.

Electromyography of the temporalis and masseter muscles. By J. D. B. MacDougall and B. L. Andrew. *University College, Dundee*

Using paired surface electrodes, it was found possible to obtain a sufficiently accurate 'electrical image' of the underlying muscles during various jaw movements. A quantitative assessment of the amount of electrical activity over different points of the head and face allowed patterns of activity to be distinguished. The masseter muscle was found to be more dominant than the temporalis during incisor occlusion, while both muscles were more or less equally involved in molar occlusion. Repeated observations at a number of test spots, each overlying a well-defined muscle component, were also made. The results of this part of the investigation confirmed the usual account given of the actions of the temporalis and masseter muscles. In addition, large action potentials were demonstrated over these muscles towards the end of maximal mouth opening. This activity preceded the onset of pain in the region of the mandibular joint and ramus by a small time margin, and was probably an indication of a protective mechanism designed to prevent dislocation of the joint.

The effect of interruption of pelvic arteries on the viability of the urinary bladder in the female rat. By J. L. Braithwaite. *University of Liverpool*

The normal distribution of arteries to the urinary bladder has been investigated in six rats.

Experiments involving the ligation of individual pelvic arteries and combinations of these were carried out in fifty-six animals, the type of operation varying from the occlusion of a single vesical artery to bilateral section of the common iliac arteries. The effect on the bladder was studied histologically and the morphology of the collateral circulation investigated by arteriographic methods and dissection.

Following some bilateral procedures atrophic changes are evident in the bladder wall, being most pronounced in the epithelium. The collateral circulation is established primarily by means of fine vessels and only after a latent period of about a week do the preformed arterial channels become noticeably enlarged. The most constant of these in the more extensive operations are the spermatic, lumbar, ureteric and rectal anastomoses.

The motor cell groups of the spinal cord in vertebrates.

By G. J. Romanes. *University of Edinburgh*

The motor cell groups in the enlargements of the spinal cord are poorly developed in Amphibia, Reptilia and Aves, despite the complex nature of the limbs which they supply. It is only in the Mammalia that these structures are highly differentiated and here the motor cell groups are remarkably constant despite functional and anatomical differences in the limbs. In a few mammals there is an unusual simplicity of these structures in the lumbosacral part of the spinal cord. This is associated with a normal arrangement of the groups in the cervical enlargement and bears no obvious relationship to structural modifications of the hind limb. The significance of these facts is discussed.

The anatomy of 'organized' nerve endings in human skin.

By G. Weddell and D. C. Sinclair. *University of Oxford*

We have recently found that in the pinna of the human ear, where all four customarily recognized 'modalites' of sensation are represented, 'organized' nerve endings are probably completely absent. The conclusion was drawn that the von Frey theory of cutaneous sensation cannot be of universal application to all areas of human skin. It still remained possible that the idea of morphological specificity of nerve endings was valid for those regions in which 'organized' endings are known to occur. Accordingly, silver-impregnated portions of skin removed at operation or post-mortem from the finger, nipple, lip, labium majus, clitoris, glans penis and foreskin have been examined. In all these regions numerous 'organized' endings have been found, but in none is there any evidence of absolute morphological boundaries between one type of ending and another. There is, rather, a 'spectrum' of endings, one type merging into another, with many intermediate forms. Nevertheless, it is quite possible to distinguish one region from another on neurohistological grounds. A series of photomicrographs illustrating the gradual transition from simple naked endings to the most complicated encapsulated forms are presented. It is submitted that an arrangement of this kind is ill-adapted to subserve, by virtue of the specific morphology of its component endings, four well-defined modalities of sensation. This view accords with that of Ruffini, and runs contrary to that of von Frey.

The nerve supply of the levatores costarum. By A. B. Morrison.

Queen's University, Belfast

In current descriptions the levatores costarum muscles are said to be innervated from the anterior primary rami, through the corresponding intercostal nerves, by fine branches which pass through the posterior intercostal membranes and the posterior fibres of the external intercostals. Careful dissection of human material has demonstrated that each of the levatores costarum muscles is innervated by a branch from the lateral division of the posterior primary ramus. The nerve of supply enters the dorso-medial surface of the muscle. No branches from the intercostal nerves passing through the posterior intercostal membrane have been encountered in the dissections. Microscopic examination of similar material confirms the findings of gross dissection. The levatores costarum muscles are grouped with the ventro-lateral musculature by most writers. This nerve supply from the posterior primary rami, and not from the anterior rami as usually described, would indicate that they belong to the dorsal musculature, and not to the ventro-lateral musculature.

'Lipase' in the alimentary tract. By B. F. Martin. *University College, Cardiff*

Using a modification of Gomori's (1945) histochemical technique for demonstrating sites of 'lipase' activity in tissue sections, a study of six animal species (dog, cat, rat, mouse, guinea-pig and rabbit) has been made, employing Tween 40 as substrate. The tissues investigated were salivary glands, oesophagus, stomach, pyloro-duodenal junction, small intestine, caecum, colon, liver, gall bladder and pancreas.

In spite of some notable individual variations in enzyme content at certain sites, the distribution pattern of the reaction in each species was found to show fairly good regularity.

Whilst all species gave a positive reaction in liver and pancreas, there were striking species differences in the results obtained with the other tissues.

Of particular interest was the finding of a positive result in certain mucous secreting cells, and in the epithelial cells lining the ducts of some glands.

It is appreciated that in reporting results obtained with Tweens as substrates, the term 'lipase' has to be used somewhat guardedly, since it has been claimed that non-specific esterases (enzymes which are normally active towards short chain fatty acid esters) may be capable of hydrolysing the long chain Tweens. Therefore, when applying here the term 'lipase', one is perhaps really referring to what should, until the position is clarified, be labelled more non-committally 'Tween-splitting enzyme'.

Distribution of alkaline phosphatase in the mucosal cells of the small intestine of the cat. By F. R. Johnson and J. H. Kugler. *University of Sheffield*

Among the investigators who have demonstrated histochemically the presence of alkaline phosphatase in the mucosa of the intestine, only a few have observed the bilaminar distribution of this enzyme in the apical regions of the mucosal cells. As a bilaminar localization of the enzyme might have considerable physiological importance, the present study was undertaken to determine if this phenomenon was a constant feature. Each stage of the Gomori technique for demonstrating alkaline phosphatase was carefully controlled and it was found that the final step, in which the sections are placed in ammonium sulphide, was the critical one for demonstrating the presence of a bilaminar distribution of the enzyme.

Sections of the small intestine of the cat were fixed in 80 % alcohol, incubated for different times in sodium-β-glycerophosphate at pH 9·4 and, during the final stage of the technique, placed in ammonium sulphide solutions of different 'maturity' and strengths for varying times.

With varying techniques, it was noted that the alkaline phosphatase always appeared first in a bilaminar distribution and that, on leaving the sections in the ammonium sulphide for a longer time, the clear zone between the two layers became obliterated with resultant appearance of a single layer. The rate at which the two layers converged to form a single layer depended on the strength of the ammonium sulphide and on its 'maturity'. Increasing the strength increased the rate of closure between the layers, whilst increasing the 'maturity' had the opposite effect. Frequently, at the time when the two layers were in the process of being replaced by a single layer, a third layer became visible between them. Increasing the period of incubation in the substrate merely caused an increase in intensity of the stain.

The significance of these findings was discussed.

The postnatal development of the prostate and seminal vesicle in the rat. By P. L. H. Davey. *St Mary's Hospital Medical School, London*

The post-natal development of the prostate and seminal vesicle has been investigated in rats from birth to 12 weeks of age by means of histological examination and by recording the combined weight of the organs at weekly intervals.

In the normal animal the graph of weight increase with age is S-shaped, with the sharpest rise between the 5th and 9th weeks of life. Histologically, the period from birth to 6 weeks old is associated with proliferation of alveoli and maturation of epithelial cells. From 6 to 12 weeks of age secretion begins to appear in the alveoli and is abundant by 12 weeks, when the organs have an adult appearance.

Following castration at 2, 3 and 4 weeks of age in different groups of animals, the weights show an initial slight rise followed by a fall. Histologically this is associated with continued, though retarded, proliferation and maturation of epithelial cells, but secretion does not appear. After about 6 weeks of age the cells lose their high columnar appearance and there is an increase in fibrous stroma.

Following testosterone injections the weights of the organs increase rapidly as compared with the normal. This is associated with the early appearance of abundant secretion within the alveoli.

The endocrinological implications of these findings are discussed.

Observations on the adrenaline and noradrenaline content of foetal adrenal glands and para-aortic bodies. By R. E. Coupland. *University of Leeds*

Extracts of human foetal adrenal glands and para-aortic bodies have been prepared by Holton's method (1949, *J. Physiol.* **108**) and injected intravenously into chloralosed cats. Simultaneous recordings of contractions of the nictitating membrane and changes in blood pressure were made and compared with those produced by the injection of known quantities of adrenaline and noradrenaline.

The results obtained indicate that the para-aortic bodies of six specimens examined (sizes, 100 mm. to full term) contain a pressor principle which is 100 % noradrenaline, whilst the adrenal glands during the same period contain a mixture of 50 % adrenaline and 50 % noradrenaline.

Extracts of adrenal glands obtained from seven subjects of ages varying between 5 months and 55 years of age show that the noradrenaline content of these structures is reduced during the first 3 years of life to the adult level of 10–25 %.

The findings suggest that the adrenal glands and para-aortic bodies of human foetuses are functionally active from an early stage of intra-uterine life and support the histochemical observations reported by the writer to this Society in November, 1951.

Pigmentation in rodents. By R. L. Holmes. *University of Leeds*

The basis of cutaneous pigmentation in rodents is the pigmentary dendritic cell. In the mouse and rat these cells lie mainly in the upper dermis, and are associated with numerous non-dendritic dopa negative pigment cells. Dendritic cells in close association with the epidermis are relatively few, and epidermal pigmentation is slight compared with that of the dermis. In the guinea-pig and rabbit, ear skin shows intimate contact between dendritic and epidermal cells, and dermal pigmentation is absent or minimal.

Body skin of the rat and mouse shows no epidermal pigment apart from that associated with the hair follicles. Guinea-pig body skin shows typical dendritic cells in the basal epidermal layer. In all three types no dermal pigment is present.

Subcutaneous injections of trypan blue into lightly pigmented mice failed to reveal any phagocytic properties in cells of the upper dermis. This is discussed in relation to pigment acquisition by dermal cells.

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